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SPECIAL LISTING

CURRENT CANCER RESEARCH

on

SELECTED ASPECTS OF CANCER-RELATED PROTEINS, CARBOHYDRATE, LIPID, AND OTHER BIOCHEMISTRY

NOVEMBER 1981

U.S. DEPARTMENT OF HEALTH AND HUMAN SERVICES
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CURRENT CANCER RESEARCH

I. BIOCHEMISTRY OF CELL SURFACE PROTEINS

A. CHEMICAL COMPOSITION AND PHYSICAL CHARACTERISTICS OF CELL SURFACE GLYCOPROTEINS

1. MEMBRANE PROTEINS OF LEUKEMIC CELLS

Dixma, J. J., de Jong, J. G., Dekker, A. W., State University Hospital, Dept. of Haematology, *Catharijnesingel 101, 3511GV Utrecht, Netherlands*

The purpose of this study is the identification, isolation and characterization of membrane proteins associated with non-lymphoid leukemic cells.

In a previous study it was found that SDS-PAGE patterns of membrane preparations from granulocytes and leukemic myeloblasts were different with respect to some ectoproteins labelled by the periodate - NaB3H4 or galactose oxidase - NaB4H3 method. Two labelled proteins (MW 220.000 and 200.000) were found in SDS-PAGE patterns of leukemic myeloblast membrane preparations that were not present in those of granulocytes. These leukemia associated proteins will be characterized with respect to their molecular weight, sugar components, and amino acids. Their relation to the differentiation stage of the cell and the cell cycle will be investigated. Presence of these proteins on other haematopoietic cells and shedding of these proteins from leukemic cells will be examined.

Methods have been developed for the labelling of ectoproteins from granulocytes and leukemic myeloblasts. Plasma membrane enriched fractions were isolated. SDS-PAGE patterns of plasma membrane enriched fractions from labelled granulocytes and leukemic blasts were obtained. Two radioactive proteins, MW 220,000 and 200,000, were found to be present in patterns of leukemic myeloblasts and not in granulocyte patterns.

2. PURIFICATION AND CHARACTERIZATION OF LEUKEMIA-ASSOCIATED GLYCOPROTEINS

Jalanko, H., Helsingin Yliopisto, Dept. of Bacteriology & Immunology, *Fabianinkatu 33, SF00290 Helsinki, Finland*

The aim of the study is to purify and charcterize leukemia-associated cell surface glycoproteins and study their expression in vivo and in vitro.

When leukemia patients are treated with cytotoxic agents, up to one kilogram of blood cells may be destroyed during the first few days. This leads to massive proteinuria and the proteins in urine largely originate from the destroyed cells. Urine of the leukemia patients thus serves as a rich source of leucocyte-associated glycoproteins.

For this study about 50 samples of urinary proteins have been collected from leukemia patients. When studied by two-dimensional gel electrophoresis, the pattern of protein spots is clearly different in the urines of leukemia patients as compared to those of normal controls (unpublished results). To detect those proteins which come from leucocytes, antibodies against different kinds of extracts of leukemia cells and normal leucocytes have been produced. With these antibodies it is possible to precipitate those glycoproteins which originate from leukemia cells. Screening of the urines from leukemia patients with these antibodies is in progress.

The next step will be to purify the leukemia-associated glycoproteins wih affinity chromatography and with other methods used for purification of proteins (gel filtration, IEF, etc.). After purification a radioimmunoassay will be set up and the expression of these proteins will be studied both in vivo and in vitro.

3. GLYCOPROTEINS OF NORMAL/MALIGNANT HUMAN BLOOD CELLS

Gahmberg, C. G., Andersson, L. C., Vuopio, P., Jokinen, M., Karhi, K., Helsingin Yliopisto, Dept. of Bacteriology & Immunology, *Fabianinkatu 33, SF00290 Helsinki, Finland*

We have developed techniques to specifically radioactively label carbohydrate portions of cell membrane glycoproteins and glycolipids. By applying such methods followed by polyacrylamide gel electrophoresis and autoradiography, we have previously shown that purified populations of normal human blood leukocytes have characteristic and distinguishable surface glycoprotein patterns which clearly correlate with the stage of cellular maturation and activation and that various cultured benign and malignant leukocyte cell lines can be characterized by their surface glycoprotein profiles. We have recently shown that the human leukemia cell line K562 is erythroid and can be induced to red cell differentiation in vitro. In this proposal we intend to: 1) isolate and characterize surface glycoproteins characteristic for subpopulations of normal human blood leukocytes. For these studies we have a massive supply of normal human leukocytes (buffy coats from 70000 blood units a year); 2) by radiolabeling of surface glycoproteins of malignant leukocytes from patients with leukemia or lymphoma further refine methods for accurate classification according to cell type of origin and stage of differentiation and to identify surface proteins associated with certain types of hematopoietic malignancies; 3) characterize the specific changes in the surface glycoprotein expression of the human erythroleukemic cell line K562 during induced erythroid differentiation, and study the biosynthesis of well-defined erythrocyte components; 4) further establish the usefulness of surface glycophorin A as a marker for early erythroid differentiation in acute leukemia.

4. MINORITY BIOMEDICAL SUPPORT

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Work will continue on the identification of the composition of the oligosaccharide component of blood group specific glycoproteins. Purified hydrolytic enzymes will be used as a tool to help eludicate structural composition of these blood group specific compounds.

Experiments will be completed on the effect of pH and certain denaturants on the fluorescence of serum albumins. An attempt will also be made to quantify the affect of aggregation on the fluorescence of human serum albumin.

There will be a continuation of the screening for possible effective systemic insecticides. New insecticides will be introduced into the program as soon as manufacturing companies will release products for study.

The corresponding porphyrins of the pyridine carboxaldehydes and thiazole carboxaldehyde will be prepared.

Heme-free globin from fractionated and unfractionated embryo and chick hemolysates will be subjected to ion exchange chromatography and polyacrylamide gel electrophoresis. The fractionated globin chains will be subjected to enzymatic digestion and the amino acid content analyzed.

Study will continue on a series of hydrolytic enzymes and the influences of the environment of the kinetic properties of the reacting system. Design and construction of interfaces between cassett data recorders and existing laboratory equipment will be continued.

5. GALACTOPROTEIN A AND B IN NORMAL AND TRANSFORMED CELLS

Hakomori, S., Fukuda, M., Sekiguchi, K., Fred Hutchinson Cancer Research Center, *1124 Columbia St., Seattle, Washington,* 98104, United States of America

A unique glycoprotein, present in normal fibroblasts but absent in transformed cells was revealed by cell surface label with galactose oxidase-NaB3H4, and termed 'galactoprotein a' (Gap a). In contrast, the label in one glycoprotein increased significantly in transformed NILpy cells, and termed 'galactoprotein b' (Gap b). A similar glycoprotein present in various non-transformed fibroblasts and deleted in various transformed cells has been observed in various laboratories and variously termed LETS, Zeta, SF-A, CSP, etc. The Gap a increased greatly on cell contact, at G1 phase of the cell cycle, and interacted with Con A, RCA, fibrinogen and collagen. The Gap b fraction of NILpy cells contained a glycoprotein whose mobility on gel electrophoresis was faster than Gap b and called Gap bT which is regarded as transformation-specific glycoprotein.

This application is to continue the study of isolation and characterization of Gap a, Gap b, Gap bT, or the related cell surface molecule in hamster NIL or rat NRK cells and their transformed counterparts. 1) Chemical characterization of glycopeptides, oligosaccharides and peptide fragments from Gap a and b, 2) structural modification of the molecules associated with cell contact, cell cycle, and virus-induced transformation, 3) the mechanism of the interaction of Gap a and b on cell surface components and 4) structural difference between Gap a present on cells and those in serum (CIg).

The Gap a and b may play important roles in determination of cellular interaction, adhesion, and expression of transformed phenotype.

6. STRUCTURE AND FUNCTION OF THE 170K GLYCOPROTEIN

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A heterogeneous cell surface glycoprotein termed 170K glycoprotein is detectable by cell surface or metabolic labeling on various normal fibroblast cell lines. The 170K glycoprotein is absent or decreased in transformed cells, and exhibits a cell-cell contact dependent accumulation on the surface of normal fibroblasts. The heterogeneity of 170K is due to the presence of at least two physically similar glycoproteins termed procollagen and GP170. GP170 is antigenically related to the major cell surface, transformation sensitive glycoprotein, Gap a (fibronectin). One component of the 170K glycoprotein complex interacts with intermediate filaments, and may play a role in communication of information from the cell surface to the cytoplasm via the cytoskeleton. The following research application proposes to investigate: 1) the similarities and differences between GP170 and Gap a in terms of carbohydrate and protein content, 2) possible mechanisms for the interconversion of GP170 and Gap a, 3) which component of 170K interacts with intermediate filaments and the control for the interaction, 4) the basis for the cell surface accumulation of 170K glycoprotein and other membrane glycoproteins as a result of cell contact.

7. CELL SURFACE STRUCTURE AND ONCOGENIC TRANSFORMATION

Hynes, R. O., Massachusetts Inst. of Technology, Center for Cancer Research, Dept. of Biology, *77 Massachusetts Ave., Cambridge, Massachusetts,* 02139, United States of America

We propose to analyze the structure of fibronectin, a major cell surface glycoprotein which is affected by oncogenic transformation. We will analyze numbers and locations of disulfide bonds, sulfhydryl groups and carbohydrate side chains and their participation in binding sites on fibronectin for gelatin, fibrin, proteoglycans and glycosaminoglycans and in the biological activities of fibronectin. We will particularly analyze interactions of fibronectin with proteoglycans and glycosaminoglycans.

We will also study biosynthesis of fibronectin and proteoglycans, as well as C3 component of complement. All of these are made by the same cells. We will study the effects of transformation, growth regulation, and hormones on these processes.

These studies will provide information on the alterations in cell surface structure associated with transformation, the molecular bases for these alterations and the biological implications of these alterations. They will also provide insight into the mechanisms of biosynthesis of specific cell surface and secreted glycoproteins.

8. STUDY OF BASEMENT MEMBRANE GLYCOPROTEIN AND COLLAGEN

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Basement membrane components, collagen and non-collagenous glycoproteins, can be isolated in a soluble form from murine and human tissues or cultured cells by methods not expected to chemically modify the isolated components. A glycoprotein component specific to epithelial basement membranes can be obtained from murine kidney or from the medium in which murine teratocarcinoma cells are grown by extraction with buffers. Characterization of these glycoproteins indicates common antigenic properties and amino acid compositions, but differences in sugar composition. These glycoproteins will be digested with nonspecific proteolytic enzymes, and the resultant small glycopeptides will be analyzed biochemically. Immunologically identifiable basement membrane glycoprotein isolated from human tissues will be biochemically characterized and compared. Basement membrane components of both human and murine origin will be recombined in order to study the mechanisms involved in the formation of the macromolecular complex which is morphologically identifiable as basement membrane. The mechanisms of synthesis of the oligosaccharide portion of epithelial basement membrane will be studied in an in vitro system in which murine teratocarcinoma cells will be grown in chemically defined medium. Under these conditions, the oligosaccharide chain is not synthesized. The approaches to this problem will include manipulation of the available monosaccharide pool, examination of the effects of vitamin A and assessment of the role of dolichols in the synthesis of the oligosaccharide.

9. MEMBRANE GLYCOPROTEINS DURING HEPATOCARCINOGENESIS

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Chemically induced hepatocarcinogenesis is accompanied by the emergence of new cell populations exhibiting defective control of cellular proliferation and positional order, a phenotypic expression that presumably involves qualitative or quantitative alterations of cell-surface macromolecules, including the plasma membrane glycoproteins. The objective of this research is to delineate the molecular basis of qualitative differences in the plasma membrane glycoproteins of normal and malignant rat liver cells, focusing on alterations in their composition, structure, topography and dynamics. Cell populations to be studied include hepatocytes, primary and transplantable hepatocellular carcinomas and focal, proliferative lesions associated with hepatocarcinogenesis. Hepatocarcinogenesis in the ACI rat, induced by 2-acetylaminofluorene and diethylnitrosamine, will be used as the experimental model. Cells will be radiolabeled using methods specific for cell-surface glycoconjugates, e.g., oxidation with NaIO4 or D-Gal oxidase followed by reduction with NaB3H4, procedures that label sialic acids and D-Gal/D-GalNAc residues, respectively. Radiolabeled glycoproteins will be solubilized with nonionic detergent (Nonidet P-40) and resolved by polyacrylamide gel electrophoresis and affinity chromatography using carrier bound lectins. The major plasma membrane glycoproteins will be characterized with regard to their extractability from the membrane, liability to protease, primary structure as determined by mapping of tryptic peptides, and immunological cross-reactivity. Immunocytochemical techniques, using monoclonal antibodies prepared against selected glycoproteins, will be employed to assess the topographic and dynamic aspects of the integration of glycoproteins into the plasma membrane. Qualitative alterations of the plasma membrane glycoproteins will be correlated with the carcinogenic

process, malignant transformation and selective processes involved in tumor progression.

10. GLYCOPROTEIN PATTERNS IN GASTRIC CARCINOMA

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OBJECTIVE: The objective of the investigation is to study the glycoprotein patterns of surface epithelial cells in gastric carcinoma and chronic gastritis in order to increase the knowledge of the molecular pathogenesis of these diseases. In addition, the possible diagnostic value and suitability for screening purposes of glycoprotein analysis of gastric juice will be evaluated.

APPROACH: Gastric biopsies which have been taken in connection with upper gastrointestinal endoscopy will be analyzed for surface glycoproteins and glycopeptides. Biopsies are taken from the tumor, the transitional area, and from tumor-free corpus and antrum. Controls will be age- and sex-matched patients with varying degrees of chronic gastritis. In addition, gastric juice from these subjects will be analyzed for glycoproteins in the same way that the biopsy specimens are analyzed.

PROGRESS: The patient material has already been collected and the analyses of the biopsy samples are in progress. A preliminary report has been published (P. Maury: Monosaccharide patterns of endoscopic gastric biopsies in chronic gastritis and cancer, Scand. J. Gastroenterol. (1980)), in press.

11. STRUCTURE OF THYROID CELL SURFACE GLYCOPROTEINS

Edge, A. S., Harvard University, Boston Campus, School of Medicine, Dept. of Biological Chemistry, *25 Shattuck St., Boston, Massachusetts*, 02115, United States of America

The objective of the proposed research is to determine the detailed structure of three glycoproteins from the cell surface of thyroid cells. The potential role of these molecules as thyrotropin receptors will also be assessed.

The central physiological role played by plasma membrane components of thyroid cells is apparent due to the importance of regulation of the endocrine function of the thyroid by circulating molecules. Although several studies have implicated plasma membrane glycoproteins and glycolipids as thyrotropin receptors, the precise nature of the molecules responsible for this activity has not been studied.

The approach to be taken involves purification of the glycoproteins using lectin affinity chromatography, followed by isolation and analysis of individual oligosaccharides and glycopeptides derived from the glycoproteins. A combination of chemical and enzymatic techniques will be used toward this end, and the detailed structure of the oligosaccharides including branch points, position of substitution and anomeric configurations will be studied. The activity of the glycoproteins as thyrotropin receptors will be ascertained in a competitive binding assay, by assaying the effect of the glycoproteins on specific binding of (125I) thyrotropin to thyroid plasma membranes.

12. VITAMIN A AND GLYCOPROTEINS OF SKIN TUMORS

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Work is continuing in two directions: The isolation and characterization of a glycoprotein secreted into the medium upon incubation of mouse skin papillomas when treated with retinoic acid. A different glycoprotein is secreted from carcinomas. The second approach is the kinetics and mechanism of the induction of fibronectin in mouse skin papillomas when treated with retinoic acid, accompanied by the suppression of ornithine decarboxylase and collagenase induction by the carcinogen.

13. SIALOGLYCOPROTEINS IN THE CLINICAL MANAGEMENT OF HUMAN NEOPLASIA

Silver, H. K., Cancer Control Agency of British Columbia, Dept. of Advanced Therapeutics, *2656 Heather St., Vancouver, British Columbia*, V5Z 3J3, Canada

OBJECTIVES: a) To identify and characterize human tumor-associated sialoglycoproteins in cancer patients' sera and tumor cell culture. b) To evaluate the clinical significance of sialoglycoproteins as monitors of tumor burden.

APPROACH: We have demonstrated that measurement of serum sialoglycoproteins, as reflected by total serum sialic acid (N-acetyl neuraminic acid), is a tumor marker applicable to a broad spectrum of tumor types. We have also found that tumor-associated sialoglycoprotein can be partially separated from acute-phase reactant sialoglycoprotein by relatively simple biochemical fractionation. We will use this as a basis for a refined sialic acid assay and compare our investigations with previous clinical studies. We will concurrently evaluate individual sialoglycoproteins by higher resolution fractionation procedures and polyacrylamide gel electrophoresis.

14. SURFACE P-GLYCOPROTEIN AND DRUG RESISTANCE IN MAMMALIAN CELLS

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OBJECTIVE: One class of mutants selected for resistance to colchicine in Chinese hamster ovary cells displays a wide-ranging pleiotrophy of cross-resistances and collateral sensitivities to a variety of unrelated compounds and chemotherapeutic drugs. These mutants have been characterized as membrane-altered and a presumptive altered gene product, the P-glycoprotein, has been identified on the cell surface. We propose to study these mutants in more detail since it appears that this type of mutation is relatively common and is observed in different cell systems including human tumor lines. Moreover, the mechanism by which the pleiotropic phenotype is mediated is fundamental to our understanding of membrane functions in mammalian cells.

APPROACH: Toward this objective, we propose four areas of investigation. These include: (1) purification and characterization of the P-glycoprotein; (2) production of monoclonal antibodies against mutant specific surface antigens; (3) purification and amplification of DNAs coding for the P-glycoprotein; (4) DNA-mediated transfer of the drug-resistant phenotype.

15. SURFACE MEMBRANES IN NORMAL AND CANCER CELLS

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About 50% of the glycoprotein N-asparagine linked carbohydrate groups of chicken and human fibroblasts are of the oligommanosyl (high mannose) type. These molecules are found in the cell surface with carbohydrate exposed to the cell environment. In both species there are at least 5 different oligommanosyl glycopeptides whose quantity and specific structure show marked alterations with malignancy and cell growth status. We propose to purify these substances in chemical amount from cells and from Sindbis virus and Semliki Forest virus grown in cells in various physiologic states (density inhibited rapidly growing, cancer cells). We will determine structures using high field proton magnetic resonance spectroscopy, gas-liquid chromatography, methylation analyses and specific glycosidase digestion.

A related but different project is to study the biosynthesis and assembly into membrane of specific plasma membrane glycoproteins and nonglycosylated proteins. 5'-nucleotidase, Sindbis virus E1 and E2 glycoproteins and vesicular stomatitis virus M protein will be studied.

BIBLIOGRAPHIC REFERENCES: Atkinson, P.H. and Hakimi, J. (1980). Alterations in glycoproteins of the cell surface. In: Biochemistry of Proteoglycans and Glycoproteins, W.J. Lennarz, ed., Plenum Publishing Corp., N.Y. pp. 191-239. Hakimi, J. and Atkinson, P.H. (1980). Growth dependent alterations in oligomannosyl glycopeptides expressed in Sindbis virus glycoproteins. Biochemistry, in press.

16. GLYCOPROTEINS FROM CANCER CELLS

Codington, J. F., Massachusetts General Hospital, Medical Services Laboratory, *32 Fruit St., Boston, Massachusetts*, 02114, United States of America

Studies of the physicochemical properties of epiglycanin will be continued. Its masking role at the cell surface will be further investigated in relation to its immunochemical and immunological properties. The relationship of the masking mecha-

nism to the type and location of the carbohydrate chains on epiglycanin will be further studied by the process of mapping active sites by electron microscopy. The biosynthesis of epiglycanin will be studied by precursor labeling and by the use of the epiglycanin antibody. Investigation of possible inhibitors of the synthesis of this glycoprotein will be pursued.

BIBLIOGRAPHIC REFERENCES: Codington, J. F., Yamazaki, T., Van den Eijnden, D. H., Evans, N. A., and Jeanloz, R. W. Unequivocal evidence for a beta-D-configuration of the galactose residue in the disaccharide chain in epiglycanin, the major glycoprotein of the TA3-Ha Tumor cell. FEBS Lett 99: 70-72, 1979. Codington, J. F., Klein, G., Silber, C., Linsley, K. B., and Jeanloz, R. W. Variations in the sialic acid compositions in glycoproteins of mouse ascites tumor cell surfaces. Biochemistry 18: 2145-2149, 1979.

17. ELECTRON MICROSCOPY OF MACROMOLECULAR STRUCTURES

Slayter, H. S., Sidney Farber Cancer Center Inc., *44 Binney St., Boston, Massachusetts*, 02115, United States of America

Inadequate specimen contrast, the effects of dehydration and beam damage limit application of the electron microscope to problems in biopolymer structure. The proposed research will continue to develop high resolution specimen-preparation methods, applying new or recently-improved techniques to systems of biological and/or clinical interest. The latter include: cell surface glycoproteins, muscle proteins, blood proteins (including immune-system components) and bronchial mucins.

The proposed research is divided into three parts: (a) Optimization of specimen preparation by improvement in fine-grain metal replication techniques developed in this laboratory; (b) A study of the degree and effects of hydration of small globular proteins by comparison of measurements on both air-dried and lyophilized electron microscope preparations with parameters measured in solution; (c) Applications to specific problems of biological and potential clinical importance will include: (1) Mapping of structural features by means of group-specific markers (such as lectins or IgG, complexed with the glycosidic side chains of glycoproteins. Subjects of mapping experiments will include epiglycanin (a mammary tumor cell surface glycoprotein which has been related to metastatic potential), fibrinogen, myosin, complement component Clq. (2) Determination of size, shape and conformation of other macromolecular systems including bronchial mucins, fibronectin (cold insoluble globulin), complement component Clq (with particular reference to the evolutionary origin of this structurally distinctive macromolecule), and hamster female protein (apparently a new member of the 'pentraxin' group which includes cross reactive protein and the P-component of amyloid deposits).

18. ROLE OF THE CELL SURFACE IN CANCER AND DEVELOPMENT

Brown, J. C., Klotz, K. L., Patterson, C. L., Garg, L. C., University of Virginia, School of Medicine, Dept. of Microbiology, *Box 246, Charlottesville, Virginia*, 22901, United States of America

A focused study is proposed here on the protein and glycoprotein components of a model mammalian cell membrane, the mouse L cell plasma membrane. These membranes will be prepared in three different ways and characterized morphologically by electron microscopy and biochemically by determining their relative content of protein, phospholipid and cholesterol. The protein and glycoprotein components associated with the three membrane preparations will be identified by their electrophoresis mobilities on SDS-polyacrylamide gels. Further characterization of individual membrane proteins will involve preparing peptide 'fingerprints' and comparing them with similar fingerprints of known L cell proteins such as actin, myosin and tropomyosin. Individual membrane proteins will also be distinguished according to whether and under what circumstances they can become phosphorylated in vivo and in vitro.

More detailed studies will be carried out on one particular L cell surface glycoprotein, a 100,000 MW Con A receptor called RHO. Structural studies in purified RHO will be undertaken to determine the relative content of carbohydrate and polypeptide in the overall molecule. Also, antiserum directed against RHO will be employed to identify cross-reacting antigens recently found to be present on the surface of mouse T lymphocytes. The antiserum will also be employed to test directly whether RHO may be involved in the transport of nutrients into L cells.

BIBLIOGRAPHIC REFERENCES: Nagpal, M.L. and J.C. Brown (1980). Protein and Glycoprotein Components of Phagosome Membranes Derived from Mouse L Cells. Int. J. Biochem. 11: 127-138. Kew, D., C. Kaznowski and J.C. Brown (1979). Preparation and Characterization of Phagosome Membranes from Arbacia punctulata embryo cells. Biol. Bull. 157: 375.

19. THE SURFACE MEMBRANES OF NORMAL AND CANCER CELLS

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Comparison of the membrane glycoproteins of control and malignant cells has revealed a difference in their bound carbohydrate groups. The difference which occurs in the sugar groups of over 80% of the glycoproteins in all the membrane systems of the cell is very widespread occurring in five species of cells in solid tumors and malignant cells in culture. The significance of this finding is unknown because aside from a few well known dramatic examples of involvement of bound sugars in specific function, the basic role of bound sugars is not really known. We have formulated an hypothesis which may answer the question why proteins bear carbohydrates. Experiments that may provide some answers to this question of protein bound carbohydrates are proposed.

We also plan to complete comparative studies to determine the structure and the biosynthesis of protein-bound carbohydrates of control and transformed cells. We will continue studies determining the amino acids of specific glycoproteins that are glycosylated. Is the 'wrong' amino acid glycosylated in the malignant cell?

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20. CELL SURFACE GLYCOPROTEINS

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We are interested in the structures and functions of carbohydrate components of cell membranes. Our systems include normal and transformed cells, and murine cell synthesizing free and membrane-bound immunoglobulin.

The transformants include malignant and non-malignant cells. The major cell surface change which gives the best correlation with tumorigenicity can be detected by two-dimensional electrophoresis of glycoproteins or Sephadex chromatography of glycopeptides. We have isolated a cell glycoprotein of 100,000 D which appears to be a cell membrane antigen involved in tumor regression.

IgM producing lines include fetal hybridomas producing mu chains only, B cell tumors producing membrane IgM, and myelomas and hybridomas producing soluble IgM. We are also initiating studies in lines producing IgG and IgA. Our studies include detailed chemical characterization of the structures of oligosaccharides at each glycosylation site on the various forms of IgM. We are also initiating studies on the biosynthesis of complex polysaccharides, and in particular how the subcellular systems can determine various carbohydrate structures on the same glycoprotein.

21. TUMORIGENESIS AND A CELL SURFACE GROWTH INHIBITOR

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We propose to characterize the physical-chemical properties and the biological activity of the cell surface components that we have isolated from mouse cerebral cortex tissue that are capable of inhibiting cellular macromolecular synthesis and replication. Our present data on the inhibitor, purified over 3000-

fold, indicates that the inhibitor is a cell surface glycoprotein. Several primary, tissue culture adapted, and tumorigenic cell lines will be used to determine why highly malignant cells are relatively insensitive, or completely refractory to the inhibitory action of the cell surface substance. After further purification by affinity chromatography and isoelectric focusing we will obtain an antibody and prepare radiolabelled monovalent F(ab) fragments. This will provide a sensitive probe to detect and measure the relative amounts of this substance on nontumorigenic and tumorigenic cells. Radiolabelled inhibitor and F(ab) fragments will also be used to measure the quantity of inhibitor bound to sensitive and insensitive cells and to identify the site of action at the subcellular level. Our investigation will include experiments on the phase of the mitotic cycle where the inhibitor arrests cell replication and to pursue our preliminary data that suggests cells eventually escape growth inhibition.

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22. CELL SURFACE COMPONENTS IN CANCER CELLS

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OBJECTIVE: The glycoprotein gp70 is both an endogeneous plasma membrane comment and the major envelop commonent of C-Type retroviruses. This project will correlate possible changes in the amino acid sequence of cell-surface gp70 during in vivo transformation of murine lymphocytes by C-Type retroviruses.

APPROACH: The AKR stain of mice are chronically infested with a C-Type retrovirus which results in the development of thymic lymphoma during older age. Trypticpeptide mapping of surface-labeled cells will be used to correlate possible changes in gp70 during cellular transformation. Further characterize the involvement of gp70 in during leukemogenesis, purified viral isolates with altered gp70's and known oncogenicities will be injected into mice, followed by a search for these altered gp70's in the resulting lymphoma cells.

PROGRESS: Tumors derived from uv- irradiated mice have been used for the production of syngenetic antisera to the tumor specific antigens (TSA's). One of these TSA's found on the surface of 1591 cell line has been identified as the viral envelope component gp70.

23. GENETIC STUDIES OF GLYCOPROTEINS AND TRANSFORMATION

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This is part of a broader project. A summary of this subproject is not available.

24. INTERACTION OF LIMULUS LECTIN WITH MEMBRANE GLYCOPROTEINS

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This is part of a broader project. A summary of this subproject is not available.

25. BIOCHEMICAL AND IMMUNOLOGICAL ANALYSIS OF MAMMARY CELL SURFACE (RATS)

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This is part of a broader project. A summary of this subproject is not available.

B. BIOSYNTHESIS AND METABOLISM OF CELL SURFACE GLYCOPROTEINS

26. GLYCOPROTEIN CHANGES DURING DIFFERENTIATION OF AVIAN ERYTHROID CELLS

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Chicken erythroblasts infected with a temperature-sensitive mutant (ts 34) of avian erythroblastosis (AEV) synthesize hemoglobin when shifted from permissive temperature (35 degrees C) to non-permissive temperature (41 degrees C), suggesting that the virus causes leukemia by blocking the differentiation of the target cells. Since the viral transforming protein may be active at the plasma membrane, the differentiation has been further studied by looking for changes in glycoprotein synthesis after growth of infected cells at 41 degrees C. Using 35S-methionine-labelled cells, affinity chromatography and immune precipitation (antisera from H. Beug, DKFZ, Heidelberg and R. Boyd, University of Innsbruck, Innsbruck) it was found that at 41 degrees C there was a marked decline in the amount of several lentil-bound glycoproteins, especially those precipitated by antisera against chicken erythroblasts, yolk sac, foetal liver or immature lymphocytes. A 90000 dalton, lentil-bound glycoprotein, present at both 35 degrees C and 41 degrees C was precipitated by anti-erythrocyte sera. The disappearance of non-erythroid-specific antigens was accompanied by a marked increase in synthesis of a 96000 dalton, ricin-bound glycoprotien (G96) which could also be precipitated by anti-erythrocyte sera. Using 125I-labelling, mature erythrocytes were shown to possess at least 4 ricin-binding glycoproteins, the two major ones having MW of 96000 and 90000 (approx.) daltons. In addition, mature erythrocytes were found to have few lentil-bound glycoproteins. Further experiments are now being carried out to determine if these ricin-bound glycoproteins are on the cell surface and whether they are related to those found in erythroblasts. Experiments are also planned to investigate the biosynthesis of the 96000 dalton molecule during erythroblast differentiation.

27. STRUCTURE, BIOSYNTHESIS AND ROLE IN DIFFERENTIATION OF MEMBRANE GLYCOPROTEINS

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The long-range goal of this project is the structural and functional characterization of cell membrane glycoproteins. Previous results from many laboratories including ours have demonstrated that plasma membrane preparations from many different sources show multiple glycopeptide bands when examined by SDS-gel electrophoresis. An understanding of the chemical nature of individual proteins will allow us to define structural, antigenic, and genetic relationships among these proteins. It will be of help to understand the significance of multiple glycoprotein components in these membrane systems. It will furthermore provide the sound basis for studies on the assembly of membranes during biosynthesis, on post-translational modifications, and their role for differentiation and on structural changes which may occur in the transformed state. The overall goal of this project will be approached by extending previous observations through a series of specific problems: I. Define more precisely the chemical and immunochemical nature of the different sialoglycoproteins in human erythrocyte membranes. II. Isolate and characterize sialoglycoproteins from red blood cells of mouse, sheep, and horse erythrocyte membranes. III. Develop procedures and analyze membrane glycoproteins from nucleated cells in various tissues. IV. Define more precisely the conformation and nature of the interaction of glycophorin A subunits. V. Investigate the biosynthesis of red cell membrane glycoproteins in an in vitro system. VI. Study the sialoglycoproteins in a differentiating system.

28. ERYTHROLEUKEMIA CELL DIFFERENTIATION

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The proposed project is a combined biochemical and genetic study on the expression of erythrocyte membrane functions during the induced differentiation of mouse erythroleukemia cells (MEL cells) in culture. Erythroid differentiation is promoted in these cells by addition of the inducing agent dimethylsulfoxide. The overall goals of the project are (1) to determine to what extent the MEL cell membrane comes to resemble that of the mature erythrocyte as a result of in vitro differentiation and (2) to determine whether the expression of red cell membrane functions is controlled separately or coordinately with expression of the genes coding for hemoglobin. Biochemical studies on the expression of red cell membrane functions are designed to determine whether red cell membrane proteins such as glycophorin and the band 3 protein (the anion channel) are synthesized in differentiating MEL cells and, if so, when they are synthesized relative to each other and relative to the time of hemoglobin synthesis. Complementary biochemical studies are intended to determine whether pre-existing MEL cell membrane proteins are modified or selectively degraded during the course of in vitro differentiation.

The genetic studies to be carried out in this project are devoted to determining whether expression of differentiated functions in MEL cells is controlled by one or by a series of several regulatory events. These studies will involve isolating genetic mutant or variant MEL cell lines which have lost the ability to synthesize hemoglobin because they are defective in the regulatory step(s) controlling hemoglobin gene expression. These 'white' clones will be examined for the synthesis of certain red cell membrane proteins, including spectrin and glycophorin. If differentiation is controlled in a single step then one expects that cells defective for the regulation of hemoglobin synthesis will also be unable to synthesize red cell membrane proteins.

29. PLASMA MEMBRANE SYNTHESIS IN FRIEND LEUKEMIC CELLS

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Some of the events that take place during the differentiation of human and mouse erythroleukemic cells include an initiation of the synthesis of two membrane proteins; spectrin and glycophorin, a decrease in lectin-mediated agglutinability, changes in surface morphology and an asymmetric division into a nucleus-containing cell and a vesicle containing hemoglobin and erythrocyte membrane proteins.

We shall investigate the mechanism of these changes. The initiation and subsequent cessation of membrane protein synthesis will be examined by isolation of the messanger RNA (mRNA) coding for these proteins, and its translation in vitro. We shall be looking for changes in the mRNA levels during differentiation.

During translation it is thought that many membrane glycoproteins are synthesized as precursor molecules with molecular weights greater than the final product. Using our in vitro protein synthesis system and specific antisera, we shall investigate whether this is the case for erythrocyte membrane glycoproteins.

The mechanism of the asymmetric division of erythroleukemic cells will be investigated by electron and fluorescence microscopy of differentiating cells. We shall be looking for cytoskeletal structures that may be involved in the production of the vesicle. The effects of various drugs on the vesicle will be examined in an attempt to elucidate the mechanism of the sequestration of erythrocyte membrane proteins into this structure.

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30. HIGH MOLECULAR WEIGHT, EXTRACELLULAR MATRIX GLYCOPROTEINS SYNTHESIZED BY ENDODERM CELLS

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Work has continued on the characterization of the two extracellular matrix glycoproteins synthesized in large amounts by the teratocarcinoma-derived parietal endoderm cell line PYS-1. The proteins, called PYS A and B, have molecular weights of 350K and 220K daltons, respectively, on SDS gels under reducing conditions. They are resistant to collagenase and are immunoprecipitable by rabbit antiserum raised against teratocarcinoma embryoid bodies but not by antifibronectin antibodies. The possibility is being tested that A and B are the same as the recently described basement membrane glycoprotein, laminin, which is synthesized as a 400K dalton precursor and cleaved to two 200K components (Timpl et al., Journal of Biological Chemistry, in press). Normal parietal endoderm cells isolated from 10 1/2-day mouse embryos synthesize large amounts of A and B and incorporate them into the underlying basement membrane (Reicherts' membrane). Isolated visceral endoderm and mesoderm cells produce very little A and B and no synthesis can be detected in undifferentiated embryonal carcinoma stem cells. A and B are being used as biochemical markers with which to follow in vitro the differentiation of teratocarcinoma stem cells into parietal endoderm.

31. STRUCTURE, BIOSYNTHESIS AND FUNCTION OF GLYCOPROTEINS

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We have demonstrated that the synthesis of the major complex-type oligosaccharide unit of glycoproteins is initiated by the en bloc transfer of a high molecular weight lipid-linked oligosaccharide (LLO) (Glc3Man9GlcNac2) to the nascent protein. This oligosaccharide is then processed by the removal of glucose and mannose residues and the addition of outer sugar residues to give rise to the mature oligosaccharide. We have also elucidated the complete structure of the LLO and the major processing intermediates and have proposed a scheme for the sequence of processing. We now propose to determine the sequence of mannose and glucose addition during the synthesis of the LLO. Cells will be labeled in vivo with radioactive sugar precursors and the biosynthetic LLO intermediates will be isolated and their structures determined. Preliminary results suggest that sugar addition is highly ordered. We will attempt to isolate and characterize the biosynthetic enzymes using LLOs of known structure as acceptors. We will also attempt to isolate minor species of LLOs which may serve special functions, such as being precursors of oligosaccharides of unusual structure. To facilitate these experiments, we will attempt to isolate new lectin-resistant mutants that have specific blocks in the synthesis of the major LLO and therefore must utilize minor pathways for glycosylation. We plan to isolate and characterize the glucosidases and mannosidases which carry out processing. We postulate that the conformation of the protein determines whether the protein-bound oligosaccharide is partially processed (i.e., to a high mannose-type oligosaccharide) or completely processed (i.e., to a complex-type oligosaccharide). To study this, we will incorporate amino acid analogs into the glycoproteins of enveloped viruses and immunoglobulins in an attempt to alter protein conformation and then determine whether processing of the oligosaccharide units of these molecules is altered. We will also compare the behavior of native glycoprotein to that of glycoprotein containing amino acid analogs when glycosylation of both has been inhibited with tunicamycin.

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32. BIOSYNTHESIS OF GLYCOPROTEINS IN NORMAL AND TRANSFORMED CELLS

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On the basis of currently available evidence, glycosylation of proteins containing asparagine-linked carbohydrate chains includes three separate molecular events: (a) preassembly of a large oligosaccharide precursor on the lipid carrier dolichol

pyrophosphate, (b) transfer of the completed oligosaccharide (Glc3Man9GlcNAc2) to nascent polypeptides and (c) processing of the protein-bound oligosaccharides by removal of the glucose residues and possibly some mannose residues to form mature high mannose and complex units. Left unanswered is the extent to which the structure and levels of the intermediates of glycosylation vary within a cell type and the consequences of such variations. There is reason to believe that most of the variability in the structures of asparagine-linked oligosaccharides (as observed in cell surface glycoproteins of normal cells and the transformed counterpart) may be based on differences in the mannosyl core region of oligosaccharides, synthesized via lipid intermediates.

To determine whether viral transformation alters the intermediates of glycosylation, a rat embryonic fibroblast system will be used. This system includes 3 cell lines: (1) normal Rat 1 cells formerly called F2408 cells, (2) Rat 1 cells transformed by the avian sarcoma virus B77, and (3) Rat 1 cells transformed by a temperature-sensitive (ts) mutant of avian sarcoma virus B77. The latter cell manifests a transformed phenotype at the permissive temperature of 34 degrees C and a nontransformed phenotype at the nonpermissive temperature of 41 degrees C. The effect of transformation on the glycosylation pathway in the rat cell system will be investigated by examining the synthesis and structure of oligosaccharides derived from (1) lipid-linked intermediates, (2) protein-bound intermediates in the processing mechanism, and (3) mature glycoproteins. The lipid-linked and protein-bound intermediates will be analyzed qualitatively by in vivo studies and quantitated with the aid of radioactive sodium borohydride. In vitro experiments will be conducted to determine the biochemical basis for any significant variations in the carbohydrate intermediates. Chemical characterization of the saccharides will be obtained by conventional methods of carbohydrate analysis.

33. BIOSYNTHESIS, PROCESSING AND ACCUMULATION OF FIBRONECTIN IN NORMAL AND TRANSFORMED CELLS

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The levels of fibronectin, a major cell surface glycoprotein of many normal animal cells, are greatly reduced in transformed cells. The rate of synthesis of fibronectin in transformed cells is depressed but not to a degree that is sufficient to account for the full decrease in surface levels. A thorough analysis of the biosynthesis of this glycoprotein is necessary to understand why transformed cells make less fibronectin and bind and metabolize what they do make quite differently from normal cells. The approach outlined here will explore the relationship of the rate of cellular proliferation to specific changes in the rates of fibronectin syntheisis and degradation. Factors such as serum concentration, cell density, and the effect of specific hormones will be tested in normal cells in order to obtain baseline information with which to compare the transformed cells.

The processes of biosynthesis of fibronectin in transformed cells will be analyzed. Aspects of the maturation and assembly of newly synthesized fibronectin such as the addition of carbohydrates and their subsequent processing and the formation of disulfide bonds will be examined and compared with these processes in normal cells. The results will reveal whether there are any qualitative differences between the fibronectin made by normal and transformed cells which could possibly explain reduced levels of fibronectin observed in transformed cells.

34. GLYCOPROTEIN SYNTHESIS AND FUNCTION DURING THE CELL CYCLE

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Strong correlations exist between changes in the compositional and physical properties of the cell surface and the proliferative activity of cells. Reports dealing with normal cells (at mitosis and stimulated to proliferate by various means), transformed cells and differentiating cells support this conclusion and indicate that alterations in cell surface carbohydrate structures, probably in the form of glycoproteins, are instrumental in mediating these changes in proliferative behavior. Differences in surface carbohydrate structure have been demonstrated between growing and non-growing cells. Virally transformed cells also appear to exhibit their major differences in surface protein structure at the level of their carbohydrate constituents. Finally, the only biochemically defined cell division cycle mutant in mammalian cells manifests its lesion at the stage of protein glycosylation. Nonetheless, the precise molecular alterations involved in these situations remain to be defined.

It is the thrust of this proposal to investigate the relationship between surface expression of specific carbohydrate types and the cell division process. The two approaches employed will be: 1) stimulation of cell division followed by rigorous analyses of the glycoproteins that subsequently are synthesized at various cell cycle stages; 2) specific inhibition of protein glycosylation followed by anlyses of the susbsequent ability of the cells to proliferate.

35. MURINE ASCITES TUMOR CELL GLYCOPROTEINS

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This research proposal is designed to study the biochemistry of alpha-D-galactopyranosyl units in animal glycoproteins. We shall investigate the mode of biosynthesis, biodegradation and possible biological significance of this relatively rare carbohydrate group. Our model system will be the Ehrlich ascites tumor cell, the membrane of which contains a family of glycoproteins with alpha-D-galactosyl end groups. Alpha galactosyltransferase activity has been localized in the microsomal fraction of an Ehrlich cell homogenate. The alpha-glactosyltransferase(s) will be purified and studied with regard to its (their) physical-chemical and kinetic properties. Acceptor activity of the transferase(s) will be studied using a recently developed solid phase assay system.

We will also assay for the activity of sialyl transferase(s) using solid phase oligosaccharide acceptors to determine whether a deficiency in this enzymatic activity leads to alpha-galactosylation.

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36. THE BIOSYNTHESIS OF CELL ENVELOPE GLYCOPROTEINS

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Cell surface glycoproteins have been shown to undergo qualitative and quantiative changes during cellular transformation and during the growth phases of cells in culture. They have been implicated as being involved in cellular adhesion, cellular migration and aggregation, and in cell-cell communication. We propose to investigate the reactions involved in the biosynthesis of glycoproteins in mammalian tissues. Oligosaccharide-lipid intermediates of glycoprotein synthesis will be isolated from liver and will be utilized as acceptors for studies on glycosyltrnsfer reactions. More specifically we intend to: a) Isolate physiological acceptors for mammalian mannosyltransfereases; b) Solubilize and purify membrane-bound mannosyltransferases; c) Characterize the purified glycosyltransferases in regard to mechanism, specificity, and kinetic properties; d) Identify the pathways by whcih the oligosaccharide chains are assembled; e) Determine whether metabolic controls regulate glycoprotein biosyntehsis at the level of glycosyltransferases or direct the specificity of glycosyltransferases.

Results from these investigations will elucidate the mechanisms involved in glycoprotein biosynthesis and will allow a clearer understanding of the role glycoproteins play in cell transformation, regulation and development, in intercellular communication, and as part of the extracellular matrix.

37. CELL AND VIRUS GLYCOPROTEINS - SYNTHESIS AND FUNCTION

Robbins, P. W., Ivatt, R., Snider, M., Rosner, M., Narasimhan, S., Huffaker, T., Massachusetts Inst. of Technology, Center for Cancer Research, *77 Massachusetts Ave., Cambridge, Massachusetts*, 02139, United States of America

Work in our laboratory is primarily in the area of glycoprotein synthesis. We have made the rather surprising discovery that a single lipid-linked oligosaccharide serves as the general precursor of all asparagine-linked glycopeptides. We have studied the pathway of synthesis of this lipid linked oligosaccharide and have examined the regulation of its synthesis by pulse-chase experiments. We have also investigated the intracellular site of synthesis of the oligosaccharide and the site of its transfer to nascent polypeptide chains. In addition, we have considered the processing reactions that occur to glycoproteins following their synthesis. Known glycoprotein processing reactions include polypeptide chain cleavage, addition of fatty acyl groups, and processing of oligosaccharide carbohydrate. Finally, changes in glycosylation patterns associated with expression of particular cell surface antigens, with developmental changes in Dictyostelium, and with transformation have also been investigated.

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38. BIOSYNTHESIS OF GLYCOPROTEINS IN NORMAL, MUTANT AND TRANSFORMED CELLS

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This is part of a broader project. A summary of this subproject is not available.

39. LIPID INTERMEDIATES IN MEMBRANE GLYCOPROTEINS BIOSYNTHESIS

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This is part of a broader project. A summary of this subproject is not available.

40. ACTION OF ANTICANCER DRUGS ON ENERGY METABOLISM AND MEMBRANE GLYCOPROTEINS OF CANCER CELLS

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OBJECTIVE: To describe the effects of anticancer drugs on the metabolism in cancer cells by studying glycolysis, respiration and membrane glycoproteins.

APPROACH: Various cancer cell lines are grown in vitro (L1210, K562, KB). The drugs studied are used at concentrations close to their ID50. The energy metabolism is studied by measuring glytolytic flux, cellular and mitochondrial respiration, AMP, ADP, ATP, and glycolytic enzymes. The alterations in membrane glycoproteins are described after mild trypsin hydrolysis and two-dimensional electrophoresis. The action on membrane transport functions is also evaluated.

PROGRESS: Some anticancer drugs, such as methotrexate and pyrimido derivatives, greatly alter cell respiration, glycolysis and energy balance. Alterations in glycoprotein patterns and inhibition of membrane transport for deoxyglucose, thymidine and uridine occur at the same time.

A new program is now under development to study the molecular alterations of a drug within the cell by use of Raman spectroscopy. Preliminary report: Raman Spectroscopy Congress, Ottawa, 1980.

41. THE GOLGI COMPLEX IN CELL REGULATION - CONTROL OF EXPRESSION OF CELL MEMBRANE CONSTITUENTS

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The cell surface and more specifically, integral membrane glycoproteins and glycolipids are implicated in the processes of increased growth and transformation as well as metastasis.

Fundamental studies over the past few years have documented a regulatory step at the level of the Golgi complex in the insertion and retrieval of integral membrane proteins to and from the cell surface. This proposal aims at genetically dissecting out the structural-functional correlates of saccules and vesicles in the Golgi complex by studying the biogenesis of several mutants of vasicular stomatitis virus in various cultured cells. The object is to attempt to provide a morphologic-genetic map of regulation at the level of the Golgi complex.

The studies will be applied to membrane proteins of oncoviruses and other integral membrane glycoproteins correlated with tumorigenicity. It is hoped the fundamental knowledge accrued from proposed studies can be eventually turned to therapeutic advantage.

42. MEMBRANE BIOGENESIS IN NORMAL AND MALIGNANT CELLS

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The objective of the proposed research is to utilize a genetic approach in combination with a biochemical, a cell biological, and a cell-engineering approach to examine the mechanisms involved in the regulation of plasma membrane biogenesis, primarily in normal but also in malignant cells. The system that is being studied consists of two membrane glycoproteins, denoted D and K, whose structures are specified by the major histocompatibility locus of the mouse, the histocompatibility-2 region on chromosome 17. Lactoperoxidase-catalyzed iodination and sodium borotritide reduction of cells treated in situ with glactose oxidase are used to label the D and K proteins accessible on the plasma membrane of macrophages, lymphocytes, and hepatocytes of different inbred mouse strains. Metabolic labeling with fucose, mannose or methionine is used to label the total cellular complement of these proteins in the different strains. Cell fractionation methods, in combination with the differential labeling techniques, are used to localize and quantitate the D and K glycoproteins among the different membrane systems of the mouse cells. The D and K glycoproteins are being isolated from these membrane systems, using specific allo and monoclonal antibodies; they are analyzed and quantitated by two-dimensional polyacrylamide gel electrophoretic techniques. Using these types of analyses, we have shown differential expression of these two H-2-encoded polypeptides in (1) different tissues of the same mouse strain and (2) in the same tissue of different mouse strains. The genetic and biochemical basis for these differences will be examined. Attempts will also be made to differentially modulate the D and K polypeptides in order to trace the intracellular localization and route of biogenesis of the perturbed glycoprotein. As part of these studies, attempts are being made to alter the expression of the D and K polypeptides and to change the histocompatibility-2 phenotype of mouse cells by (1) transferring specificities from one cell to another, using membrane reconstitution and fusion methods and using gene transfer techniques. The expectation is that the above approaches will yield important insights into the genetic control of expression of these membrane glycoproteins, their mode of biogenesis and turnover, and their metabolic function within the cell.

43. DOLICHYL PHOSPHATE BIOSYNTHESIS IN TUMOR CELLS

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Dolichyl phosphate is known to act as a lipophilic cofactor in mediating the transfer of oligosaccharides to proteins. It has been suggested that the levels of this molecule may be limiting in the glycosylation of nascent polypeptide chains. De novo biosynthesis of dolichyl phosphate may modulate its cellular concentration. The proposed research will focus on the biosynthesis of dolichyl phosphate in two tumor cell lines: Ehrlich ascites tumor cells and cultured mouse L1210 cells.

It is proposed that the purification of the key enzyme of dolichyl phosphate biosynthesis in Ehrlich tumor cells, the long-chain prenyl transferase, be undertaken. The enzyme will be purified by conventional means and characterized physically. Cofactor and substrate requirements, as well as the kinetic mechanism, will also be investigated. In addition, the subcellular location and transmembrane topology will be investigated.

The induction of long-chain prenyl transferase activity in mouse L1210 cells will be attempted by two means: 1) growing cells at different cell densities; 2) causing a deficiency in dolichyl phosphate levels by means of the antibiotic compactin, which specifically blocks an early enzyme in the biosynthetic pathway. By correlating changes in enzyme levels with dolichyl phosphate levels, several types of questions may be answered: a) is the long-chain prenyl transferase rate-limiting in the synthesis of dolichyl phosphate; b) what is the rate of decline of dolichyl phosphate levels when de novo synthesis is blocked; and c) what is the effect of a dolichyl phosphate deficiency on membrane glycoprotein biosynthesis?

BIBLIOGRAPHIC REFERENCES: Adair, W.L. and Robertson, S. (1980) Absolute Configuration of dolichol, Biochemical J, 189, 441-445.

44. GLYCOSYLATION PATHWAYS IN ANIMAL CELLS

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This laboratory has previously isolated eight genetically and biochemically distinct mutants of Chinese hamster ovary (CHO) cells all of which exhibit specific defects in the glycosylation of membrane glycoproteins (and, in some cases, membrane glycolipids). They were selected for resistance to the cytotoxicity of a variety of plant lectins. A glycosyltransferase enzyme defect which appears to be responsible for one of these mutants has been localized and the altered carbohydrate moieties synthesized by this mutant have been structurally described. This mutant has provided precise and valuable information in such areas as glycoprotein biosynthesis, the specificities involved in lectin-cell surface interactions and virus-cell surface adsorption specificities. The properties of the other seven mutants suggest that these mutants belong to a family of glycosylation mutants with lesions analogous to the glycosyltransferase deficiency previously described. The aim of this proposal is to define in structural terms the nature and extent of the carbohydrate alteration in the seven lectin resistant mutants previously isolated and to search for the enzymic basis of the mutation to lectin-resistance in each case. The structural studies will be performed on the carbohydrate moieties from animal viruses which reflect the glycosylation defects of the mutant cells or on cell surface carbohydrate moieties if no virus vectors can be found. The search for enzymic lesions will involve in vitro glycosyl transfer assays using endogenous and exogenous glycosylated acceptors. Priority will be given to mutants affected solely in glycoprotein synthesis and isolated from a single-step selection protocol.

45. STUDY OF GLYCOCONJUGATES IN COLONIC NEOPLASIA

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The major objective of our proposed study is to elucidate changes in the structure and metabolism of cell surface glycoconjugates which accompany malignant transformation in the colon. We hope to achieve this objective by examining the structures of cell surface glycoconjugates using biochemical, immunochemical and immunological techniques. Following cell surface labeling, we will isolate surface components from colonic tumors, normal tissues and normal and malignant colonic cells. Those which are tumor specific will be extensively characterized. We will compare the synthetic and degradative processes associated with these tumor antigens and compare these processes with those in normal cells and tissues. The turnover and shedding rates of cell surface glycoconjugates will be examined in cultured normal and malignant cells and related to in vivo detection of tumor specific antigens in the body fluids of cancer patients and tumor-bearing animals. Using external cell surface labelling and lectins, we will study the effects differentiating agents have on cell surface components and on the growth properties of cultured cells. We will also compare surface glycoconjugates and cell-cell interactions of cells with different metastatic potential. In this connection, we will examine cell surface glycoconjugates of fibroblasts taken from patients with premalignant conditions such as adenomatosis of the colon and rectum. Finally, we plan to pursue some promising results we have obtained from studies on the effects of dietary fiber constituents on the incidence, degree of malignancy and distribution of colonic tumors in rats.

BIBLIOGRAPHIC REFERENCES: Tsao, D. and Kim, Y.S.: Separation of cell surface glycoproteins from glycolipids by Ricinus communis agglutinin-Sepharose. J. Biol. Chem. In press, 1981. McNutt, N.S., Mak, L.L., and Kim, Y.S.: Comparison of cell peripheries in the human adenocarcinoma cell lines SW 480 and SW 620 grown in floating chamber culture, coverslip culture, athymic mice and Balb/c mice. Lab. Invest. In press, 1981.

46. METABOLISM AND FUNCTION OF GLYCOPROTEINS AND GLYCOLIPIDS

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The overall objective of this research is to study the structure, biogenesis and function of animal cell surfaces. This interest has been stimulated by recent observations relating cell surfaces with regulation of cellular growth. The specific purpose of this project is to understand the mechanism(s) of glycosylation (with particular emphasis on sialic acid) of glycolipids and glycoproteins. We will continue and extend to other sugar nucleotides our current studies on the subcellular site(s) of synthesis of CMP-sialic acid. Attempts will be made to determine how sugar nucleotides get from their intracellular site(s) of synthesis to the site(s) of sugar transfer. We will further pursue our recent observations on the uptake by cells of sialic acid and the subsequent incorporation of this sugar into glycoproteins and lipids. The intracellular site(s) where the covalent binding of sialic acid to glycoproteins and glycolipids occurs will be studied by pulse-chase kinetics with cells in tissue culture and mouse liver. Although initial experiments will monitor the glycosylation of a mixture of glycoproteins and lipids, future studies will concentrate on one particular glycoprotein. The subcellular movement of covalently bound label will be monitored by a combination of subcellular fractionation and electron microscopy-autoradiography. These studies with normal cells will be extended to cells in tissue culture infected with a virus that is temperature sensitive for transformation since such a system should enable us to distinguish transformation related phenomena from ancillary ones.

47. MUTANTS ALTERED IN GLYCOSYLATION OF MEMBRANE PROTEINS

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The general aim of my laboratory's research is to delineate the various steps in the glycosylation of proteins and to determine possible biological consequences of impaired glycosylation. Our approach is to isolate and characterize mammalian cell mutants defective in glycosylation. These mutants will allow us to catalog various reactions in the synthetic pathway, and to determine what effect an alteration in a particular reaction has on different glycoprotein products. Previously we determined that both the cells and membrane preparations of a concanavalin-A resistant Chinese hamster ovary cell line (B211) failed to glucosylate its oligosaccharide-lipid intermediates and protein.

Presently, we are examining individual membrane-associated and soluble glycoproteins in this cell line. This study should help to elucidate the role of glucose in the biosynthesis of these glycoproteins and the biological consequences of this glycosylation defect.

48. ISOLATION AND CHARACTERIZATION OF MUTANT CELL LINES DEFECTIVE IN GLYCOPROTEIN BIOSYNTHESIS

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SPECIFIC AIMS: 1) to obtain lectin-resistant cell lines that have novel alteration in cell surface oligosaccharides 2) to isolate and characterize the altered cell surface oligosaccharides 3) To define the enzymatic basis for the defect in the variant cell lines.

APPROACH: Several tissue culture cell lines will be used to derive lectin-resistant clones by established selection procedure. The various lectins that will be used are Concanavalin A, Soybean Agglutinin, Ricin and Modeccin. The lectin-resistant cells will be cultured for brief periods in radioactive mannose and glucosamine and subsequently the cells will be extracted. The extracted pellets will be digested with pronase and the released labeled glycopeptides and oligosaccharides from lipid-linked oligosaccharides characterized and interesting mutations screened by a combination of lectin-affinity column elution profiles and digestion with various glycosidases. Structural studies on oligosaccharides will be done by methylation analysis, Smith periodate degradation, acetolysis and glycosidase digestions. Structural determinations of oligosaccharides from mutant cells should give an indication of possible defects, many of which can be tested in vitro. In conjuction with this vesicular stomatitis virus infected mutant cells will be used to study the effect any postulated defects may have on the synthesis of the viral glycoprotein.

49. ROLE OF COATED VESICLES IN INTRACELLULAR TRANSPORT OF GLYCOPROTEINS

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We propose to test the hypothesis that coated vesicles and coated protein (clathrin) are involved in selective binding and packaging of glycoprotein synthesized at the rough endoplasmic reticulum (RER), and undergo a directed migration which results in specific insertion of the nascent protein into the cell plasma membrane. A model system for these studies is provided by vesicular stomatitis virus (VSV) glycoprotein (G protein) maturation in infected HeLa cells. An in vivo demonstration of coated vesicle coupling to G transport will be attempted by either immuno-precipitation of vesicles by monospecific anti-clathrin antibody and analysis using SDS-polyacrylamide gel electrophoresis, or pulse-chase experiments whereby the glycosylated forms of G are shown to be associated in a sequential, transient fashion with vesicles purified to homogeneity by use of density gradient techniques. A model for coated vesicle formation will be formulated on the basis of results obtained from density shift experiments where cells grown on light (12C14N1H) amino acids are shifted to heavy (13C15N2H) amino acids. Attempts will be made to define an in vitro coupling of coated vesicles with nascent G protein by extension of the currently defined in vitro system for synthesis and core glycosylation of VSV glycoprotein in RER membranes.

50. MEMBRANE PROTEINS AND LIPIDS IN MAMMARY CARCINOMA

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The surface membrane related characteristics which distinguish tumorigenic and nontumorigenic cells derived from mouse mammary carcinomas and normal mouse mammary glands are being determined. The tumorigenic cells exhibit alterations in several classes of fucosylated compounds, namely fucosylated amino acids, trypsin-sensitive surface fucopeptides and cell surface associated fucoproteins. As an approach to understanding the metabolism of the fucopeptides the mannose and glucose analog, 2-deoxy-D-glucose, has been used. There is a differential effect on the trypsin-sensitive surface fucopeptides when normal and transformed cells are cultured in medium supplemented with 2-deoxy-D-glucose. In addition, there are small but consistent differences in the choline phosphatides of the tumorigenic and nontumorigenic mammary cells.

51. STUDIES ON TRANSFORMATION-SENSITIVE CELL SURFACE PROTEIN

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A large transformation-sensitive cell surface glycoprotein is found to be induced by cell-cell contact and distributed in cell-cell contact area. This proposal is directed toward the total understanding of the metabolism, fibrillogenesis, distribution, regulation and function of this protein.

52. STUDIES ON PROTEINS INVOLVED IN CELL INTERACTION

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This proposal is directed toward the total understanding of the metabolism, fibrillogenesis, distribution, regulation and function of major cell surface and intercellular matrix protein, fibronectin.

C. FUNCTIONAL ASPECTS OF CELL SURFACE GLYCOPROTEINS AND OTHER MEMBRANE PROTEINS

53. CELL ADHESION FACTOR - BIOLOGY AND CHEMISTRY

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A plasma membrane glycoprotein, found in the extracellular matrix of normal eukaryotes but absent following transformation, is also present in substantial quantities in normal plasma. The protein has been designated fibronectin and has been shown to mediate the adhesion of cells to a collagen substratum in vitro.

As a means of determining an in vivo function for this protein we are investigating its chemical structure and adapting the techniques of in vitro cellular adhesion to a more biologically relevant substratum, specifically basement membranes excised directly from experimental animals. These limiting membranes are known to contain substantial levels of fibronectin. It is assumed that antisera directed against fibronectin and known to inhibit cellular adhesion in vitro will provide experimental means of determining the relevance of fibronectin to cellular adhesion in vivo.

Subtilisin digestion of fibronectin under specific conditions yields reproducible patterns of digestion products. Following chromatography of the fragments on a gelatin affinity column a major polypeptide of apparent molecular weight 50,000 daltons binds to and is eluted with 4M urea from the column. This fragment is still capable of mediating adhesion of fibroblasts to collagen and therefore contains both the gelatin and cellular binding sites. Further chemical characterization of this fragment has been accomplished.

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54. STRUCTURE AND ROLE OF A TRANSFORMATION-SENSITIVE CELL SURFACE GLYCOPROTEIN

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The major cell surface glycoprotein fibronectin is decreased after malignant transformation and is involved in cellular adhesion. We have investigated its mechanism of action. Polypeptide structural domains containing specific binding sites for collagen and the plasma membrane have been isolated and characterized biologically. High-affinity, separate binding sites

for hyaluoronic acid and heparin were also characterized, and a third domain for heparin binding was identified.

The role of fibronectin in normal differentiation was examined. Cartilage cell development involves the loss of cell surface fibronectin, apparently due to a loss of receptors. Vitamin A reverses these events. Maintaining fibonectin levels inhibits cartilage cell development.

Our objectives will be to determine the composition and structure of the multiple active sites of fibronectin, and the mechanisms by which this major cell surface protein helps to regulate cell behavior and differentiation.

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55. THE CELL SURFACE BINDING OF FIBRONECTIN

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The objective of this project is to study the interactions between the cell surface and fibronectin and the molecular basis for cellular adhesion as mediated by this glycoprotein. The approach we plan involves the detailed examination of the binding of fibronectin to gangliosides by thermodynamic, kinetic and chemical methods. The binding constants of gangliosides to fibronectin will be determined by indirect methods via changes in fluorescence emission and CD spectra. In the most interesting cases direct binding studies of labeled gangliosides to fibronectin will be carried out. These gangliosides shall also be reconstituted into phospholipid vesicles to serve as a simple model system for cellular adhesion. The kinetics of reconstituted vesicle aggregation upon the addition of purified dimeric fibronectin shall be examined. The results are to be analyzed with respect to the concentrations of gangliosides and fibronectin in terms of possible mechanisms for fibronectin mediated cell-cell aggregation. The spatial relationship between cell surface gangliosides and fibronectin will be determined on cells by cross-linking experiments. We will use cleavable heterobifunctional cross-linkers to couple fibronectin to gangliosides, solubilize the membrane components, purify those coupled to fibronectin and analyze these components after cleavage of the cross-linker by either specific immunoprecipitation or thin layer chromatography of the gangliosides.

56. ANALYSIS OF FIBRONECTIN-CELL INTERACTIONS BY CHEMICAL CROSSLINKING

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Fibronectin is a major cell surface glycoprotein of many normal animal cells but levels are greatly reduced in transformed cells. It appears to be important in the maintenance of normal fibroblast morphology and adhesiveness since the addition of fibronectin to transformed cells restores normal cell shape, adhesion, and cytoskeletal organization. Fibronectin has been crosslinked to sulfated proteoglycans at the cell surface and an apparent transmembrane linkage with intracellular actin microfilaments has been reported. The nature of the fibronectin membrane association can only be ascertained by identifying the cell surface molecules with which it interacts. The approach described here may provide this information and allow further understanding of how fibronectin binding to cells affects cell behavior and cytoskeletal organization and how it can restore normal characteristics to transformed cells.

Chemical crosslinking of fibronectin at the cell surface will be used to examine the fibronectin cell interaction. Fibronectin with an attached crosslinking reagent will be bound and crosslinked to fibroblasts. Crosslinked complexes will be isolated either from total cell lysates or from a cytoskeletal framework produced by detergent extraction of cells. Crosslinks will be analyzed by polyacrylamide gel electrophoresis and immunochemical methods and components will be tested for direct interaction in vitro.

57. FIBRONECTIN - PROTEOGLYCAN BINDING IN ADHESION SITES

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The surface membrane properties of murine 3T3, malignant SV40-transformed 3T3, and revertant variants of transformed cells (revertants have regained several behavioral properties characteristic of normal cells growing in tissue culture) are being examined with attention focused on substrate-attached material (SAM), cell-substratum adhesion sites which remain bound to the tissue culture substrate after EGTA-mediated detachment of cells. SAM is greatly enriched in the cell surface glycoprotein fibronectin and certain glycosaminoglycan-containing proteoglycans. Preliminary experiments indicate that two different proteoglycan aggregates in SAM may bind to fibronectin in different ways to act as positive or negative effectors of adhesion. We will seek evidence that fibronectin in these adhesion sites can bind to various GAG-containing species in dissociated SAM and will examine the specificity and topology of this binding in SAM from the three cell types grown under various physiological conditions. Evidence will also be sought that fibronectin:proteoglycan complexes exist as 'native' components in these adhesion sites. These studies will shed further light on how these two classes of cell surface components effect adhesion of normal or malignant cells to extracellular matrices in the first place, secondly how detachment of cells to generate 'footprints' during motility of cells is effected by one type of supramolecular proteoglycan aggregate, and finally how normal malignant cells may differ in their adhesive processes.

58. CELL SURFACE STRUCTURE AND CELL TRANSFORMATIONS

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We will carry out a coordinated cell biological and biochemical analysis of protein-protein interactions at the cell surface and their role in several related cellular functions. Our particular focus will be on the glycoprotein fibronectin, and its interactions with other proteins both outside the cell and across the membrane with actin microfilaments. Fibornectin is involved in adhesion, spreading and migration of fibroblasts and is reduced in amount by oncogenic transformation. Fibronectin has also been implicated in the interaction of platelets with collagen and as an opsonin in phagocytosis. We will investigate the involvement of fibronectin in these phenomena by functional assays, immunofluorescence, and electron microscopy, and will study its interactions with other proteins by chemical crosslinking reagents and affinity chromatography. This study will provide information about multimolecular interactions at the cell surface and transmembrane connections with the cytoskeleton and their involvement in several important cellular and physiological properties (oncogenic transformation, adhesion, migration, hemostasis, phagocytosis).

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59. CELL ADHESION PROTEINS AND MALIGNANCY

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The goal of this project is to examine the role of fibronectin in cell adhesion. The proposal is aimed at a structural and functional characterization of fibronectin.

We have prepared a large number of monoclonal antibodies to human fibronectin. We will continue our characterization of these monoclonal antibodies. We intend to determine the nature of the antigen recognized by each of the monoclonal antibodies. We will also test each monoclonal antibody for its ability to inhibit one or more of the biological activities of fibronectin.

With the aid of monoclonal antibodies, we will map the structural gene for fibronectin to a human chromosome.

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60. THE MOLECULAR BASES OF CELL ADHESION

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Alterations of the adhesive interaction between cells and components of the interstitial matrix such as collagen are doubtlesaly important in the invasive and metastatic behavior of malignant cells.

Excepting the rapidly growing body of knowledge concerning fibronectin, little information exists on the molecular bases of cell-matrix interaction. Clearly, surface macromolecules other than fibronectin are involved in adhesion, as are components of the cytoskeleton, but biochemical details of these involvements are scanty. Moreover, the effects of clear cut alterations of in vitro adhesiveness on the invasive or metastatic behavior cells in situ or in vivo are unknown.

The present proposal intends to explore the molecular basis and biological consequences of altered cell-substratum adhesiveness using membrane biochemistry and somatic cell genetics as tools. We have isolated and partially characterized a series of variant clones of CHO cells which are defective in their ability to adhere to a collagen substratum; we suspect that the defect may be due to an altered surface binding of fibronectin. We intend to investigate the molecular aspects of adhesion by comparing the membrane biochemistry of the variant clones with that of wild type CHO clones. We will examine the surface glycoproteins, glycosaminoglycans and glycolipids of the membranes of both types of cells. We will also examine the role of cytoskeletal sol-gel transitions in the adhesion of both wild type and variant cells. In addition we will study the biological behavior of wild type and adhesion variant CHO cells in terms of invasiveness in the chick chorioallantoic membrane and metastasis formation in nude mice.

61. MECHANISMS OF NORMAL AND NEOPLASTIC CELL ATTACHMENT

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Despite the importance of cell-substratum interactions in determining the behavior of normal and neoplastic cells, the proteins responsible for attachment have not yet been identified directly. We have developed a method whereby lactoperoxidase is covalently coupled to polystyrene tissue culture flasks and used to radioiodinate the monolayer cell proteins that come into intimate contact with the lactoperoxidase-polystyrene surface.

In the proposed project, this system would be used to label the polypeptides in extracellular material and plasma membranes of attached cells. Lactoperoxidase will also be coupled to various natural extracellular proteins and used to label the membrane and, possibly, the intracellular proteins that interact with it. The labeled polypeptides will be analyzed on exponential gradient SDS-polyacrylamide gels and their localization will be determined by usual fractionation methods.

The data thus obtained should reveal the chain of interactions between extracellular, intramembrane and intracellular proteins in normal cells. Disturbances that accompany neoplastic transformation should also be apparent.

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62. SURFACE MEMBRANE GLYCOPROTEINS INVOLVED IN THE MAINTENANCE OF CELLULAR ADHESION AND MORPHOLOGY

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Previous studies in this laboratory have revealed consistent, reproducible changes in the glycopeptide composition of surface membranes from transformed cells when compared with non-transformed, control cells. These changes involve most of the glycoproteins from every membrane system of the cell. They may well be responsible for the altered behavior and invasive properties of the transformed cell. However, before the significance of such alterations can be understood, the glycoproteins involved in such fundamental properties as cellular adhesion and morphological determination must be identified and characterized. Towards this end, a series of antisera have been developed which induce reversible rounding and detachment of the cell from its substratum. Using these antisera, we propose to: (1) identify the cell surface components involved in the maintenance of adhesion and morphology, (2) produce monospecific, monoclonal antibodies against those purified components using the hybridoma system, (3) isolate and characterize cellular mutants containing alterations in these surface components, and (4) begin detailed biochemical analysis of these individual components. These studies should allow our previous biochemical observations to be put in biological perspective.

63. ARRANGEMENT OF LETS PROTEIN ON CELL SURFACES

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The LETS protein is a peripheral membrane protein found on the surface of mammalian cells. It is lost upon transformation, and appears to be involved in cell adhesion to artificial substrate or collagen, cell-cell interactions, cytoskeleton structure, and control of cell growth. The aim of this research is to understand these functions by determining with which proteins LETS associates on the cell surface, and by investigating the nature of the associations.

The associated proteins will be identified by chemical crosslinking. Purified LETS will be reacted with one end of a reagent whose free end contains a photosensitive group. This protein will then be added to cells and photolyzed to crosslink LETS to nearby proteins, and the products analyzed in a two-dimensional gel system. Once identified, the function, orientation, and interactions of these proteins will be studied.

64. GENETICS OF COHESIVE PROTEIN IN DICTYOSTELIUM

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I propose to investigate the specific developmentally regulated cell-cell cohesion system of the cellular slime mold Dictyostelium discoideum. I will purify a developmentally-regulated cell-surface protein known as 'contact sites A', raise monospecific antisera against the purified protein, and use antibodies purified from those antisera to identify the protein species in membrane preparations from Dictyostelium which are related to 'contact sites A'. The purified contact sites will be tested for specific binding to aggregation-competent cells as a possible assay for biological activity. The purified material will be used to identify and isolate the cell-surface species which bind to 'contact sites A'; this material in turn will be used, in conjunction with the anti-contact-sites antibodies, to isolate structural-gene mutations in the 'contact sites A' molecule using the fluorescence-activated cell sorter. Characteristics of these mutations will be determined by standard biochemical and genetic techniques and will be used to investigate the function and control of 'contact sites A' during the organism's development.

65. MEMBRANE CHANGES CAUSED BY TUMOR VIRUS TRANSFORMATION

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In an effort to elucidate the cell surface molecules involved both in the adhesion of cells to their substratum and in the interaction of skeletal elements with the surface membrane, a series of antisera have been produced which react with the cell surfaces. Two of these antisera induce reversible cell rounding and substrate detachment. The third has no effect on cell morphology or adhesion. An assay based upon the ability of membrane extracts will be fractionated and attempts made to purify the constitutuents which block the interaction of the immunoglobulin with the cells. Using the recently described methods for preparing immune-B-cell/plasmacytoma hybrids, monoclonal antibodies reactive with individual cell surface glycoproteins will be produced. These antibodies will be used for further structural, biochemical and genetic studies designed to understand the problems of cell adhesion and membrane-cytoskeletal interaction.

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66. THE BIOLOGICAL ROLE OF GLYCOPROTEIN CARBOHYDRATES

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Glycosylation of protein is not required for the export or secretion of fibronectin, acetylcholine receptor (AChR), and procollagen, nor for the fusion of embryonic muscle cells in culture. Similarly, glycosylation is not required for the biological activity of fibronectin or the alpha-bungarotoxin binding of AChR. Studies with all these systems indicated that the carbohydrate was required to stabilize the protein moiety against proteolytic degradation.

The 'complex' carbohydrate structure of fibronectin is synthesized as a 'high-mannose' precursor.

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67. BIOCHEMICAL STUDIES ON MEMBRANE BOUND IMMUNOGLOBULIN

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The modes of attachment of cell surface proteins are being studied in mouse cells, trypanosomes and viruses. The methods used will include surface iodination using 125I as the labeling agent as well as conventional methods of proteolytic enzyme digestion and subsequent structural analysis of surface proteins. The aim of this work is to study the mechanisms responsible for the interaction of the membrane and its associated proteins.

68. ROLE OF GLYCOLIPIDS AND GLYCOPROTEINS IN LYMPHOCYTE ACTIVATION

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The objectives of this project are to investigate the behavior and interactions of cell surface glycolipid and glycoprotein receptors and to determine their importance in the activation of B and T lymphocytes by mitogens such as plant lectins. Gangliosides and lectin receptor glycoproteins from lymphocytes and erythrocyte membranes will be isolated and reincorporated into lipid bilayer model membranes in controlled component ratios. Binding of radiolabelled lectins to specific receptors will be characterized and the modulation of this binding by other glycolipid and glycoprotein molecules studied. Gangliosides and receptor glycoproteins will be spin labelled by covalent modification of the carbohydrate headgroup, and ESR spectroscopy used to obtain detailed information on the headgroup mobility, environment, interaction with other receptor molecules and crosslinking by divalent cations. Spin-labelled gangliosides will be incorporated into the plasma membrane of intact lymphocytes and used as a probe of the activation process, providing data on the molecular motion and interactions of the carbohydrate headgroup. Attempts will be made to manipulate the lymphocyte activation process by incorporating 'foreign' glycolipid and glycoprotein receptors into intact cells, and monitoring enhancement or suppression of mitogenic activation. This project should provide important data on glycolipid and glycoprotein receptor behavior at the molecular level, in both lipid bilayers and cell membranes. Manipulation of the process of lymphocyte activation could prove an extremely useful immunological tool and provide further insight into the complex mechanisms occurring in vivo.

69. THE ROLE OF THE T145 GLYCOPROTEIN IN THE FUNCTION OF CYTOLYTIC T-LYMPHOCYTES

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OBJECTIVE: We want to determine if T145, a surface glycoprotein which is expressed specifically on mature cytolytic T cells, is participating in the molecular events that underlie the cytolytic activity of these lymphocytes.

APPROACH: The approach is based on somatic cell genetics and biochemical analysis of established cell lines expressing the CTL phenotype.

PROGRESS: The glycoprotein T145 has been identified on several CTL-lines and has been purified from mitogen activated T cells.

70. STUDIES OF THE MEMBRANE ORGANIZATION AND REACTIONS OF PLASMA MEMBRANE SIALOGLYCOPROTEINS THAT MEDIATE T LYMPHOCYTE ACTIVATION

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The oxidative mitogens periodate and neuraminidase plus galactose oxidase activate T lymphocytes by creating aldehyde groups on cell surface sialoglycoproteins. These aldehydes undergo further unknown reactions that ultimately lead to lymphocyte activation. Recently, it has been shown that, of all the membrane components of rat T lymphocytes modified by the oxidative mitogens, a set of four high molecular weight sialoglycoproteins (referred to as the tetrad molecules) are the ones responsible for mediating cellular activation. In addition, the tetrad molecules have been found to react with monoclonal antibody prepared by Dr. A.F. Williams against a rat leukocyte-common antigen.

I propose to investigate some aspects of the structure and function of the tetrad molecules to learn how they mediate oxidative mitogenesis. The structural studies will determine whether the tetrad molecules are transmembrane proteins. Two approaches will be used to selectively probe the cytoplasmic face of the lymphocyte plasma membrane: 1) preparation of sealed, inside-out, plasma membrane vesicles; and 2) unfolding of the plasma membrane on polylysine-coated beads.

The functional studies will determine what the aldehydes on the oxidized tetrad molecules react with and what the kinetics of these reactions are. Experiments have been designed to identify the surface components that form Schiff base adducts with oxidized tetrad molecules. Schiff bases will be selectively reduced with tritiated cyanoborohydride, and the tetrad adducts will be immunoprecipitated, hydrolyzed and analyzed by SDS gel electrophoresis. The kinetics of the reactions of tetrad aldehydes will be followed by introducing a tritium tag on the aldehyde carbon atoms. By using immunoprecipitation and SDS gel electrophoresis, the amount of free, crosslinked, and total labeled tetrad molecules will be determined as a function of time during the early hours after lymphocyte oxidation.

71. THE USE OF A BIOTIN-AVIDIN SYSTEM TO STUDY INTERACTIONS OF CELL SURFACE GLYCOPROTEINS REQUIRED FOR THE ACTIVATION OF T-LYMPHOCYTES

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No summary has been provided to the Smithsonian Science Information Exchange.

72. CELL SURFACE CARBOHYDRATE AND LYMPHOCYTE INTERACTIONS

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Cell surface glycosyltransferases are being purified and characterized from rat and bovine lymphocytes to elucidate their functioning in cell surface phenomena in these systems. Surface glycoconjugates are being labelled isotopically using chemical and enzymatic methods. A receptor from calf lymphocytes, specific for N-acetyl glucosamine is being purified and characterized. Cell interactions using various mitogens in culture systems as models are being studied.

73. STRUCTURE, SHEDDING AND BIOSYNTHESIS OF A MUCIN-TYPE CELL SURFACE GLYCOPROTEIN

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The overall goal of this program is to develop an understanding of the role(s) played by sialoglycoproteins in cell surface phenomena. In this project we are concentrating on a large, predominantly mucin-type sialoglycoprotein (ASGP-1) found in ascites forms of the 13762 mammary adenocarcinoma. ASGP-1 molecules found in xenotransplantable (MAT-C1) and species specific (MAT-B1) forms of the tumor have been purified to homogeneity and found to have essentially identical amino acid compositions, but different carbohydrate compositions. MAT-B1 ASGP-1 can be labeled with sulfate, but MAT-C1 ASGP-1 shows little labeling. Since differences in sulfation and sialic acid content suggest differences in the control of the biosynthesis of ASGP-1 oligosaccharides, we are investigating structures of the oligosaccharides released from ASGP-1 by alkaline borohydride. We are also examining the nature of the polypeptide chain by classical degradation techniques. Biosynthesis of ASGP-1 is being investigated after metabolic labeling by combined kinetic and autoradiographic studies. Information on the oligosaccharides structures should permit us to postulate control mechanisms for explaining differences between MAT-B1 and MAT-C1 oligosaccharides. These mechanistic proposals can then be tested by measuring levels of glycosyltransferases using isolated oligosaccharide precursors or by measuring levels of precursor pools of nucleotide sugars. Such studies should give us considerable insight into the molecular mechanism controlling the cell surface properties of these ascites cells.

74. GLYCOPROTEIN SHEDDING AND METASTASIS

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Glycoconjugates, primarily glycoproteins, are implicated in oncogenesis, maintenance of the neoplastic state and metastasis. 'Shedding' of tumor glycoproteins into the circulation has been suggested as playing an important role in tumor growth and/or metastasis. We propose to examine two aspects of glycoprotein shedding: first, correlation of serum glycoprotein:N-acetylneuraminic acid transferase activity with tumor burden and second, identification, characterization and kinetics of release (shedding) of tumor glycoproteins. Two rat mammary tumors will be used, the transplantable hormone-responsive R3230AC and the DMBA-induced hormone-dependent adenocarcinomas arising in situ. To correlate NANA transferase activity and tumor burden, serum activity will be measured at frequent times during a variety of selected regimens that alter tumor growth (hormone therapy, endocrine organ ablation), as well as during carcinogenic transformation of normal cells. Elevations in enzyme activity prior to appearance of carcinoma would suggest this assay as a prognostic test. Glycoprotein 'shedding' will be studied using tumors radiolabeled with fucose and/or glucosamine in vitro, transplantation of cells to animals and following the release of labeled glycoconjugates into the host's circulation. The influence of tumor burden and hormonal status on release of labeled glycoconjugates and characterization of the labeled components will be examined. Other enzymes involved in glycoprotein metabolism and proteolytic enzymes will be explored as potential markers for tumor growth and spread. Changes in serum NANA transferase activities and glycoconjugates during normal physiological alteration of the host will also be studied.

75. MITOGEN-DEPENDENT RELEASE OF A GLYCOPROTEIN BY 3T3 CELLS

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We have discovered that pure mitogens, such as epidermal growth factor and fibroblast growth factor, specifically stimulate the release - from 3T3 mouse embryo cells but not SV40-transformed 3T3 cells - of a glycoprotein with an apparent molecular weight of 34,000 daltons on SDS gels. We have designated this protein 'mitogen releasable protein' (MRP).

We shall determine the relation between the effect of mitogens on MRP release and DNA synthesis by comparing these two cellular responses to mitogen with respect to: (1) serum concentration dependency, (2) cell cycle dependency, (3) response to other mitogens and to compounds which act synergistically with peptide mitogens. To determine the relation between MRP release and transformation, we shall (1) analyze a number of transformed cells with different growth properties for MRP release, (2) determine whether transformed cells release factors which stimulate MRP release.

To determine the mechanism of MRP release and the defect in mitogen-stimulated release in SV40-transformed cells, we shall determine (1) whether increased appearance of MRP in the medium is due to increased synthesis, decreased degradation or increased release of MRP, (2) whether MRP is released from an intracellular site (secretory granules?) or from the cell surface, (3) the involvement of proteases, microtubules, divalent cations, cyclic nucleotides, anions, glycosylation, and lysosomal processing in MRP release, and examine (4) the time course of MRP appearance after mitogen addition.

We shall purify MRP and initiate studies of its physical and functional properties. We expect our studies to contribute information about proteins released by fibroblasts and to provide a basis for determining how these proteins may be involved in cellular growth control.

76. THE ROLE OF CYTOSKELETAL PROTEINS IN ENDOCYTOSIS

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I propose to look at the role of certain cytoskeletal proteins in endocytosis to determine if the different pathways which have been proposed for internalization of macromolecules (multivalent ligand-induced clustering and coated pits) are actually two different aspects of the same general mechanism. To do this, antibodies will be prepared against clathrin, the main protein component of coated pits. These antibodies will then be used in conjunction with antibodies against aminopeptidase, a cell surface protein known to undergo ligand-induced clustering and gamma-actinin, a component of the cytoskeleton and a marker for membrane-associated actin, in pair-wise double labeling experiments. Using indirect staining techniques, these antibodies will be used to visualize each of the proteins at the level of both the fluorescent light microscope and the transmission electron microscope so that a more complete picture of the internalization process can be obtained.

77. DYNAMICS OF PHEOCHROMOCYTOMA MEMBRANE GLYCOPROTEINS

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A clonal tumor cell line (PC12) has been derived from a rat pheochromocytoma. This cell line has been used as a model for neurobiologic and neuropathologic studies. The PC12 cells

display differentiated properties of adrenal chromaffin cells and, in addition, acquire further neural properties to resemble sympathetic neurons after treatment with nerve growth factor (NGF). We have used immunologic techniques to identify a NGS Induced Large External (NILE) glycoprotein on the plasma membrane of PC12 cells and rat sympathetic neurons. The NILE glycoprotein is a potential surface membrane neural marker for the following reasons: It is trypsin-sensitive; surface-exposed; present only on neural tissues and NGF-inducible.

The objectives of this proposal are: 1) to isolate and purify NILE glycoprotein using biochemical techniques such as affinity chromatography and SDS-polyacrylamide gel electrophoresis; 2) to prepare mono-specific antibodies to NILE glycoprotein either by immunizing a suitable recipient laboratory animal directly with NILE glycoprotein or isolate and further purify them from our heterogeneous antisera to rat sympathetic neurons using affinity chromatography; 3) to compare by morphological methods (immunoperoxidase, autoradiography) the topographic distribution of NILE glycoprotein (using monospecific anti-NILE antibodies as a probe) on the plasma membrane of perikarya and processes of PC12 cells and rat sympathetic neurons; and 4) to study the possible segregation and endocytosis of the surface NILE glycoprotein of PC12 cells and rat sympathetic neurons following exposure to anti-NILE antibodies and NGF (NGF-treated PC12 cells).

This study will contribute to our understanding of the mechanism of endocytosis and turnover of a highly specific, well-characterized portion of the plasma membrane of neurons.

78. CELL SURFACE CHANGES IN SPONTANEOUSLY OR SV40 TRANSFORMED MOUSE CELL LINES

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The purpose of this project is to study the cell surface membrane -how it may change in transformation to malignancy either by spontaneous induction or as induced by SV40 virus; to study the nature of tumor antigens associated with cells transformed by either SV40, chemicals, or spontaneous induction; and the relation of these antigens to gene expression and host immune mechanisms. Research is carried out on: 1) Changes in glycoconjugates, including glycoproteins and sulfated glycosaminoglycans such as heparan sulfate, and their correlation with cell growth properties in tissue culture and in vivo, in the syngeneic and in the nude mouse; 2) controls of expression and methods of purification of SV40 specific T antigens, surface antigens and tumor specific transplantation antigens (TSTA); 3) the relationship between SV40 T, surface and TSTA antigens, and their relationship to SV40 A gene expression; 4) a 56,000 MW SV40 induced but cell DNA encoded protein which is also present in embryonal carcinoma cells and in certain embryo cells; 5) study of 'spontaneous' transformation of cells in culture and of the balance between such tumorigenicity and the SV40 TSTA in families of clonal cells with known genealogy.

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79. SURFACE GLYCOPROTEINS OF TRANSFORMED CELLS

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This is part of a broader project. A summary of this subproject is not available.

80. THE ROLE OF PLASMA MEMBRANE PROTEINS IN THE REGULATION OF CELL BEHAVIOR

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The entry of insulin, epidermal growth factor, triiodothyronine and alpha2 macroglobulin into cells has been studied by video intensification microscopy and immunocytochemistry. Hormones initially bind to diffusely distributed receptors. The hormone ligand complexes then cluster in coated pits and are internalized in a specialized vesicle termed a receptosome. Entry is blocked by inhibitors of transglutaminase suggesting cellular TG is involved in the process.

BIBLIOGRAPHIC REFERENCES: Willingham, M.C., and Pastan, I. The Receptosome: An intermediated organelle of receptor-mediated endocytosis in cultured fibroblasts. Cell, in press, 1980. Willingham, M.C., Maxfield, F.R., and Pastan, I. Receptor-mediated endocytosis of alpha2-macroglobulin in cultured fibroblasts. J. Histochem. Cytochem., in press, 1980.

81. PROTEINS OF THE HEPATOMA CELL PLASMA MEMBRANE

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The objective of the proposed research is to understand how the mammalian cell regulates the concentration of the proteins that are externally oriented on the plasma membrane. Toward this objective we have labeled by external labeling methods those proteins that constitute the bulk of plasma membrane proteins in Hepatoma Tissue Culture (HTC) cells. We have analyzed by two-dimensional polyacrylamide gel electrophoretic methods the externally oriented proteins and glycoproteins of the plasma membrane. A complex set of glycoproteins is present on the plasma membrane, but these glycoproteins are not unique to this organelle. By cell fractionation methods and by enzyme probe analyses (with trypsin or neuraminidase) of intact and disrupted cells we can show the existence of a significant intracellular membrane-bound reservoir of these same plasma membrane glycoproteins. The function of this intracellular reservoir of plasma membrane proteins in HTC cells forms a significant portion of the present research proposal. These membrane glycoproteins are being isolated and purified in order to prepare specific antibodies and monoclonal antibodies in mice spleen:MOPC tumor cell hybrids. These antibodies are being used to study the route of biogenesis of these membrane proteins in cells not only growing normally in tissue culture but also after perturbation of the surface concentration of specific membrane glycoproteins by specific antibody or by hormones such as dexamethasone. The exchange of plasma membrane proteins with the proteins of the intracellular pool (via recycling) is also being analyzed. To pursue these studies, we have developed the methodology to insert radioactively-labeled biologically active purified membrane glycoproteins, including those specific receptor functions into the plasma membrane of cells which themselves do not have the specific biological function. With these methods, we have been able to alter the phenotype of the recipient cell to reflect that of the donor cell from which the specific plasma membrane receptor protein was obtained. Our expectation is that the above experiments will shed new and significant insight into the mechanism of biogenesis and function of plasma membrane proteins.

BIBLIOGRAPHIC REFERENCES: Doyle, D., Hou, E., and Warren, R. Transfer of the Hepatocyte Receptor for Serum Asialo-glycoproteins to the Plasma Membrane of a Fibroblast. J. Biol. Chem. 254, 6853-6856 (1979). Baumann, H., Gelehrter, T.D., and Doyle, D. Dexamethasone Regulates the Program of Secretory Glycoprotein Synthesis in Hepatoma Tissue Culture Cells. 1980, in press, J. Cell Biol

82. MAPPING OF THE CYTOPLASMIC FACE OF THE RBC MEMBRANE

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The objective of this project is the determination of molecular topography at the inner surface of human erythrocyte membranes. Specifically, I am interested in determining the neighbor relationships between spectrin, actin, glycophorin, band 3, glyceraldehyde-3-phosphate dehydrogenase, and the re-

cently discovered spectrin-binding protein topographical mapping will be performed using: a) fluorescence resonance energy transfer (FRET) to measure molecular neighbor relationships of proteins and lipids, b) fluorescence microscopy, using the same labels as in (a) to determine the supramolecular distributions of proteins and lipids, c) transmission electron microscopy with ferritin and hemocyanin to complement FRET and fluorescence microscopy data.

Both native and reconstituted membranes will be mapped using these techniques. Probes will include chemically labeled membrane proteins and monospecific antibodies directed against membrane proteins. Ultimately, I want to know how the interactions of peripheral components serve to modulate the mobility of integral membrane proteins and the fluidity of membranes. I expect to find specific protein-protein, and to a lesser extent, protein-lipid interactions which result in limited lateral ordering of membrane components. These experiments will contribute to our understanding of red blood cell shape maintenance and of the general phenomenon of anchorage modulation.

83. PLASMA MEMBRANE ALTERATIONS IN TRANSFORMED CELLS

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The plasma membrane plays a critical role in determining how cells react to external stimuli. Since the plasma membrane has been observed to have altered physiological responses and an altered protein composition in neoplastically transformed cells, it is important to further understand the structure of this organelle. Most of the previous studies on plasma membrane proteins have looked for qualitative or quantitative differences between transformed and untransformed cells. Since many of the protein species are the same in both transformed and normal cells, there is a strong indication that their special organization may be perturbed during transformation.

The objectives of this proposal are: (1) to detect which proteins are similar in membrane preparations from a variety of transformed and untransformed cells in culture, (2) to further develop methods for the characterization of the spacial location and interactions between membrane proteins and to compare these organizational features in transformed and untransformed cells. The methods include the use of inside-out membrane preparations which can be selectively labeled or marked on their inside, cytoplasmic, or external surface. The selective interaction of nonpenetrating polymers, the selective enzymatic labeling of the internal or external surface and the selective interaction of monoclonal antibodies, directed against defined externally located cell surface antigens, will be used to study the interactions of membrane proteins. This type of approach has been used to characterize less complex biological structures; however, the technology has improved to the level where the molecular analysis of plasma membrane structure can be initiated. These studies should provide basic insight into the structural organization and alterations in structure which can occur in cells and a better basis for understanding of cellular physiology.

84. THE ORGANIZATION OF PROTEINS IN THE HUMAN ERYTHROCYTE MEMBRANE

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We shall apply our current knowledge of the molecular composition and organization of human red blood cell membrane components to a study of their more complex functions. The first problem is the characterization of the structure and function of the cytoplasmic pole of band 3, the predominant membrane-spanning polypeptide and anion-transport protein of the erythrocyte. We shall complete our determination of the amino acid sequence of the 23,000 dalton fragment cleaved from amino-terminus of the cytoplasmic pole of band 3. We shall also extend our characterization of the binding site contained in this region of band 3 for glycolytic enzymes, hemoglobin, and band 2.1 (which couples a subjacent reticulum to the membrane).

Secondly, we shall examine the hypothesis that lateral inhomogeneities in lipid distribution accompany changes in membrane curvature, using 14C-lysolecithin as a probe. Since we have evidence that cholesterol can bias membrane curvature, we shall use cholesterol oxidase as a probe to examie the distribution of cholesterol between the two sides of the bilayer and the rate of cholesterol movement across the membrane. We shall also investigate the mechanism which converts the misshapen reticulocyte into a regular biconcave disk during its first few days in curculation.

Finally, we seek to understand the mechanisms for the formation of stable holes in unsealed ghosts, their resealing in warm saline and their loss of the capacity to reseal at low ionic strength, since these phenomerna may provide novel clues to membrane dynamics unstudied heretofore.

85. MASKING OF ANTIGENS AT CANCER CELL SURFACES

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The chemical, immunochemical, and physical properties of cell-surface glycoproteins in immunosensitive cell lines and the topographies of these lines, as visualized by scanning, transmission, freeze-etch, and freeze-fracture electron microscopic techniques, will be compared with those of immunoresistant cell lines of the same murine tumors. Studies will involve spontaneously-developed mammary carcinoma ascites cells which become immunoresistant spontaneously and those which were selected for immunoresistant characteristics; Moloney virus-induced lymphoma cells which have been immunoselected for resistance to antiserum to the Moloney virus-determined cell-surface antigen and those which have been selected for resistance to anti-histocompatibility (H-2) antiserum. Isotopically-labelled cell-surface components believed to be implicated in escape mechanisms, including tumor rejection antigens and macromolecules involved in masking or modifying antigenic activities, will be removed from the cells, purified, and studied for immunological properties and for detailed chemical structures, as determined by chemical, enzymatic, and immunochemical techniques.

86. FLAGELLAR MEMBRANE SIGNALLING

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This project is directed at examining the flagellar membrane signalling portion of the Chlamydomonas reinhardi mating reaction. Chlamydomonas was chosen as a system for the ease with which pertinent membrane fractions can be isolated, the lack of complication due to endocytosis and the usefulness of agglutination and signalling mutants. Digestion with proteolytic enzymes has proved to be a very useful tool for examining the role of flagellar surface polypeptides in the signalling phenomenon. Chymotrypsin digestion at low concentrations of enzyme causes loss of signalling ability without influencing flagellar agglutination. Since chymotrypsin digestion has provided important leads as to which membrane polypeptides are involved in signalling, other proteases, including glycosidases, will be explored for any which mimic chymotrypsin or exhibit greater specificity in their digestion targets and effects on signalling. Chymotrypsin digestion, following iodination of membrane surface polypeptides, in conjunction with SDS polyacrylamide gel electrophoresis and autoradiography will be used to determine the developmental and genetic distribution of signalling polypeptides. The topology of these polypeptides in the flagellar membrane will be examined by using reversible crosslinkers, such as dithio (succinimidyl propionate). The investigation of signalling will also involve the use of monospecific antibodies directed against membrane polypeptides which are likely to be involved in signalling, those which share with signalling sensitivity to chymotrypsin. Finally the signallig defect in the agglutination and signalling mutant imp-7 will be characterized.

87. GLYCOSYLATION OF PHOTORECEPTOR MEMBRANE PROTEINS

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Our interest is in studying the mechanism of glycosylation of membrane proteins in vertebrate photoreceptors and the possible role of this process in membrane assembly and segregation in these cells. The specific aims of this project are: 1) To analyze in detail the processing of the oligosaccharide chains of opsin and to investigate the role of this process in the assembly of opsin into membrane. 2) To characterize the major proteins of the synaptic region and to determine their carbohydrate content and to investigate the processes by which they become glycosylated. 3) The contamination of the glycosylation patterns of these populations of membrane proteins and the construction and testing of hypotheses relating possible differences to membrane assembly and segregation.

88. STRUCTURE AND INTERACTIONS OF METHYL ACCEPTING CHEMOTAXIS PROTEINS IN E. COLI

Dahlquist, F. W., Kehry, M. R., Oregon State Higher Education System, University of Oregon, Inst. of Molecular Biology, *Johnson Hall, Eugene, Oregon*, 97403, United States of America

Reversible methylation of methyl accepting chemotaxis proteins (MCP) located in the inner membrane is intimately involved in the response and adaptation events underlying chemotaxis in E. coli. The proposed research will investigate the role MCP I and MCP II play in transmembrane communication by studying their molecular structure, interactions, methylation and topography in the inner membrane. The molecular structure and topography of MCP I and MCP II will be determined by enzymatically and chemically cleaving radiolabeled MCPs into peptides, determining a linear order, and localizing the regions of the MCPs which are methylated in the cytoplasm, bind to receptor proteins in the periplasm or contact the inner membrane. Investigation of the interactions of the MCPs with other chemotaxis gene products will be approached by producing monoclonal antibodies specific for individual peptides in MCP I and MCP II. In addition, the role of multiple methylation sites in regulating the function of MCP I and MCP II will be examined by using specific antibodies to methylated peptides. Microsequence analyses of the methylated MCP peptides will determine whether these sites are different and facilitate kinetic studies of methylation and demethylation reactions.

89. CELL SURFACE PROTEINS OF POLYOMA VIRUS AND SV40 TRANSFORMED CELLS (HAMSTERS)

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This is part of a broader project. A summary of this subproject is not available.

90. CELL SURFACE GLYCOPROTEINS OF HEMATOPOIETIC CELLS (HUMAN, MICE)

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This is part of a broader project. A summary of this subproject is not available.

91. STUDIES ON TRANSFORMATION SENSITIVE CELL SURFACE PROTEIN

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This is part of a broader project. A summary of this subproject is not available.

92. PURIFICATION OF PLASMA MEMBRANES FROM PRE-IMPLANTATION MOUSE EMBRYOS

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It is widely believed that the cell surface plays a vital role in the development of the mammalian embryo. However, no specific function in embryonic cell interaction or morphogenesis has yet been ascribed to a surface component. It is our aim to follow changes in surface proteins and glycoproteins during preimplantation development of the mouse embryo, and to examine the relationship between these changes and cell differentiation. Our approach was to collect mouse embryos at the two-cell stage and to culture them in vitro, radio-labeling them at different stages by addition of 35S-methionine to the medium before isolating the plasma membranes. The amount of starting material is very small, but losses can be minimized by the addition of non-radioactive teratocarcinoma cells as carrier. The two-phase separation method of Brunette and Till (Journal of Membrane Biology, 1971, 5, 215) has been recommended for small-scale preparations, but we have found that the sucrose step-gradient method of Pilkis et al. (Biochemica et Biophysica Acta, 1974, 343, 250), with certain modifications to include protease inhibitors throughout the procedure, yields better results. Purity of the final membrane preparation was judged by electron microscopy appearance and by differential enzyme activity. Comparison by SDS-PAGE and auto-fluorography of membranes from embryos labelled at different stages has revealed several stage-specific membrane proteins. These will be further characterized and it is hoped that one or more can be shown to play a role in the developmental process.

93. NATURE AND INTERACTION OF CELL SURFACE DETERMINANTS IN EARLY EMBRYOS

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OBJECTIVE: To analyse the surface glycoproteins and proteins of the cells of preimplantation mouse embryos by surface-labelling techniques to detect changes in labelling patterns with time, differences between cell sub-populations and the effects on these parameters of agents that modify embryonic development in a variety of ways.

APPROACH: Surface labelling by iodination and reaction with isethionyl acetimidate; separation of proteins by one- and two-dimensional electrophoresis.

94. ROLE OF GLYCOPROTEINS IN SEA URCHIN DEVELOPMENT

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Normal embryonic development and morphogenesis require highly regulated cellular interactions involving intercellular adhesion. Cell surface complex carbohydrates and their receptors are thought to specify such contacts. The proposed project investigates the role of glycoproteins in changes in cell adhesive properties during morphogenetic events of the sea urchin embryo, especially during cellular ingression of the primary mesenchyme. Major changes in glycoproteins will be detected with electrophoretic and specific visualization techniques. Cell surface glycoproteins will be identified by labeling intact cells with the galactose oxidase and lactoperoxidase methods. Direct electrophoretic analysis of the glycoproteins of isolated and cultured cells of the sixteen cell embryo, as well as analysis with conjugated lectins, will be used to determine whether specific glycoproteins are unique to various populations of cells. The effect of monospecific antibody on cell adhesion and ingression will be used to determine if a specific cell surface glycoprotein is involved in such phenomena. Experiments are also proposed to distinguish among various levels at which cells modulate the expression of both glycoproteins and cellular affinities at their surfaces.

95. DIFFERENTIATION OF LEUKEMIA CELLS

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OBJECTIVE: The long-term goal of the present investigation is to understand molecular mechanisms involved in blocks in differentiation of human leukemic cells, and to apply the information in treatment and diagnosis of various forms of human leukemia. The short-term purpose of the present studies is to clarify the biological roles of cellular glycoproteins in leukemic cells.

APPROACH: Human promyelocytic leukemia cells (HL-60) and Friend virus infected murine erythroleukemia cells (MELC) in culture are the experimental systems under investigation. HL-60 will be induced to differentiate mature myeloid cells by tunicamycin, which specifically blocks synthesis of glycoprotein by lipid-linked pathway. Glycoproteins before and

after induction of differentiation will be analyzed by biochemical and immunological techniques. MELC can be induced to differentiate by various compounds, including nicotinamide and its related compounds, which inhibit poly(ADP-ribose) synthetase in vitro. With these compounds, as a tool, the significance of ADP-ribosylated protein will be analyzed in intact cells during the course of differentiation.

96. CELL SURFACE ALTERATIONS IN MALIGNANCY

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This grant project proposes to study rat intestinal epithelial cell glycosyltransferases and cell membrane glycoproteins in order to determine their role in the control of differentiation. Methods have been established which separate the differentiated villus cell from the undifferentiated crypt cell. Furthermore, intestinal plasma membranes have been purified and separated from a purified Golgi fraction. We propose to utilize these techniques to (1) analyze and characterize the changes in intestinal epithelial cell surface membrane glycoproteins associated with cell turnover, differentiation and tumor induction, (2) characterize the changes in intestinal epithelial cell surface membrane glycoprotein synthesis associated with differentiation and malignant transformation.

We will also study animal tumor models we have recently described, where growth of the tumors correlated with the appearance and levels in serum of an electrophoretically distinct peak of galactosyltransferase on polyacrylamide gels (galactosyltransferase isoenzyme II). These animal tumor models will be studied in order to determine the role of cell membrane glycoproteins and tumor glycosyltransferases in tumor growth and metastases. We propose specifically to (1) characterize the biological properties of the tumor-associated serum galactosyltransferase isoenzyme II, and (2) purify and characterize the tumor galactosyltransferases and compare them with normal tissue and the serum isoenzymes. It is hoped that these studies will further define the cell membrane changes peculiar to tumor growth and the ability of a tumor cell to metastasize.

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97. MEMBRANE PROTEINS IN TUMORS

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The objective of the project is to examine the hypothesis that cellular differentiation and malignant transformation are accompanied by expression of specific cell surface proteins. The surface proteins will be analyzed by a newly developed two-dimensional gel electrophoresis system. The final goal of the proposal during the period of grant support is to analyze and compare surface proteins of the normal and T/t locus mutant mouse embryos. The recessive t mutants of homozygous lethal type are uniquely suited for this project since: 1) they interfere with development of embyros at six specific stages, 2) they interfere with the further differentiation of ectoderm, 3) they are expressed as the surface antigens, and 4) a number of mutant strains are available. I propose to analyze proteins of sperm, unfertilized eggs, fertilized eggs and embryos. Analysis of sperm proteins is feasible by techniques available in this laboratory. However, sensitivity of protein detection needs to be improved for the analysis of proteins in eggs and embryos. An approximately 10 to the 4th power-fold increase of sensitivity seems possible by utilizing horseradish peroxidase-catalyzed reaction as follows: Proteins are tagged with biotin by reacting with succinimidated biotin. Biotin molecules are then complexed with avidin-horseradish peroxidase conjugate. Finally, horseradish peroxidase will be detected by catalytic polymerization of a radioactive substrate such as tyrosine, aniline derivatives or diaminobenzidine or its derivatives. A cell surface protein of MW 135,000 is inducible in Chinese hamster ovary cells by cyclic AMP derivatives or prostaglandin E1. This is an ideal model system for the study of rate of synthesis and turnover of surface proteins, pathway of protein expression on cell surfaces, and mode of protein-protein interaction on plasma membrane.

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98. GLYCOPROTEINS IN DIFFERENTIATION AND ONCOGENESIS

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The overall objective of this proposal is to understand the course of hematopoietic cells in terms of membrane glycoproteins and evaluate cell membrane glycoproteins as onco-differentiation cell surface marker, shared by tumor cells and normal cells at an early stage of differentiation.

Two major aspects will deal with: (1) Testing whether certain membrane glycoproteins can function as tumor-specific cell surface marker as well as specific marker for cells at an early stage of differentiation. We have analyzed cell surface glycoproteins of erythroblasts cultured in vitro and of human leukemic cell line K562 and found that some cell surface glycoproteins are expressed on both cells but not on mature erythrocytes. Further studies will be to isolate such glycoproteins, prepare antibodies specific to such glycoproteins and look at the distribution of such proteins in stem cell differentiation of normal individuals and patients.

(2) Clarifying the structural change of polyactosaminoglycan throughout the various ontogenic, differentiation and oncogenic stages. We have found that polylactosaminoglycan displays a drastic structural change during ontogenesis. These structural changes can be analyzed distinctively by newly developrd technique, cell surface labeling and endo-beta-galactosidase digestion. This technique already played a key role in understanding the cell surface carbohydrate structure of erythroblasts and K562 cells. Further experiments will be structural studies on polylactosamine and its analogues of B and 3 glycophorin to relate ontogenic change to the changes in differentiation and oncogenesis. In addition, the carbohydrate structure of a glycoprotein (Gp105), specifically present on K562 cells, will be characterized.

99. MEMBRANE STRUCTURE AND ENZYME INDUCTION

Chen, K. Y., Rutgers the State University of New Jersey, New Brunswick Campus, Douglass College, Dept. of Chemistry, *New Brunswick, New Jersey*, 08903, United States of America

The principal objective of this proposal is to study some of the biochemical processes that underlie differentiation; particular emphasis will be placed on the involvement of plasma membrane and ornithine decarboxylase (ODC). Mouse neuroblastoma cells can be induced to differentiate by cAMP. The differentiated cells lose their tumorigenicity. Increased tyrosine hydroxylase (TH) activity and neurite outgrowth are two distinct phenotypic expressions of differentiation in neuroblastoma cells. The possible involvement of membrane protein phosphorylation in mediating the effect of cAMP in cytodifferentiation will be studied. Differences in the composition of plasma membrane proteins of malignant and differentiated neuroblastoma cells will be probed by two surface labeling techniques (lactoperoxidase catalyzed iodination, and galactose oxidase catalyzed reductive tritiation), as well as by phosphorylation of purified membranes catalyzed by endogenous protein kinases. cAMP causes the induction of ODC and TH in neuroblastoma cells. These two enzymes are the rate-limiting enzymes in the synthesis of polyamines and catecholamines, respectively. The mechanism by which cAMP induces ODC, and the involvement of ODC in cytodifferentiation will be studied. The approach is to delineate the relationship between cAMP-induced membrane protein phsophorylation changes and the increase in ODC activity. The

temporal and/or causal relationship between the cAMP-induced increase in ODC activity and TH activity will be investigated. The approach is to correlate changes of the two enzyme activities under conditions where ODC activity is varied. The proposed studies should help us to understand the role of cAMP and polyamines in cytodifferentiation. In addition, knowledge on the composition of plasma membranes of malignant vs. differentiated cells should help us to gain a better insight into the understanding of tumor reversion. Our long-term goal is to be able to design drugs which specifically modulate the various biochemical processes which underlie differentiation, so that differentiation and tumor reversion can be manipulated pharmacologically.

100. THE ENVELOPE PRECURSOR OF HUMAN KERATINOCYTES

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During terminal differentiation of the keratinocyte, both in living tissues and in cell culture, extensive cross-linking of the keratins occurs and a resistant cross-linked protein envelope forms. The aim of the project will be to understand more fully the development and formation of the protein envelope. The envelope precursor has recently been purified and its amino acid composition has been determined. Further structural studies will be undertaken as the protein has an unusual amino acid composition which is markedly different from that of the final cross-linked envelope. The nature of the interaction of the precursor with the cell membrane is unknown and may depend upon protein and/or lipid components. Since modifications of the cell surface often appear during malignant transformation, alteration of the membrane architecture may affect envelope formation. Reconstitution studies using purified precursor and phospholipid vesicles will attempt to define the membrane-protein interaction. The presence of the mRNA in normal and neoplastic cells will be assayed.

101. MEMBRANE PROTEIN ANALYSIS DURING MUSCLE CELL DIFFERENTIATION

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It is proposed to characterize the membrane proteins of myoblasts during myogenesis. Differences in the patterns of plasma membrane proteins will be sought as myoblasts proliferate, line up, and fuse and myotubes form. The method for plasma membrane isolation that I will use is the aqueous two-phase polymer system (Brunette and Till 1971). The purified plasma membrane can then be analyzed both by computerized two-dimensional gel scanning (Garrels 1978) and by direct application of iodinated lectins to the two-dimensional gel (Burridge 1978). Both analytical methods have recently been developed at the Cold Spring Harbor Laboratory and have been shown to have high resolution and reproducible quantitation, making it possible to detect any minor but important changes in the plasma membrane proteins during myogenesis. In addition, antibodies against the purified plasma membrane from different stages of muscle cells will be prepared. Immunochemical analysis using crossed immunoelectrophoresis (Owen and Salton 1975) will provide more detailed information about membrane protein changes during myogenesis. Similar techniques can be used to analyze changes in plasma membrane proteins of nonfusing cell variants. I hope to find several important changes or regulatory events in the plasma membrane during myogenesis.

102. CELL SURFACE PROTEINS DURING DIFFERENTIATION (FUNGI)

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This is part of a broader project. A summary of this subproject is not available.

D. BIOCHEMISTRY OF RECEPTOR PROTEINS (EXCLUDING STEROID HORMONE RECEPTORS)

103. HYBRID PROTEINS FOR RECEPTOR-MEDIATED TRANSPORT STUDY

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The overall objective of this proposal is to develop methods for synthesis of biologically active protein-protein conjugates containing an enzyme and a cell surface receptor-binding protein for use as probes to study the mechanism of cell surface receptor-mediated protein transports. The proteins to be studied will involve plant and bacterial toxins, polypeptide hormones, lysosomal enzymes, desialylated glycoproteins and lipoproteins. Once a conjugate is synthesized its uptake through the putative receptor-binding protein-receptor interaction process and its express of biological activity intracellularly will be studied with receptor cell cultures. The information obtained from these studies will be useful for determining whether or not it is possible to utilize various receptor-mediated protein transport systems for introducing lysosomal enzymes into deficient cells for enzyme replacement therapy and toxin active fragment for cancer chemotherapy.

104. SUBUNIT INTERACTIONS OF MOUSE NERVE GROWTH FACTOR IN RECEPTOR BINDING

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The 7S nerve growth factor as isolated from the mouse submaxillary gland is a multisubunit protein complex containing zinc ion which exerts considerable metabolic influence upon interaction with its target tissues (sympathetic and spinal ganglia). The oligomer is comprised of three classes of subunits - alpha, beta, and gamma - of which biological activity, i.e., neurite outgrowth and maintenance of neuronal cells, is found solely with the beta species. The beta subunit is known to bind to a Triton solubilized material presumed to be the cell surface receptor and to a receptor of high affinity (nuclear binder). The binding of the alpha and/or gamma subunits and the binding and/or role of zinc ion have not been studied. This proposed work will use radiolabeling, chemical crosslinking, and immunochemical techniques to gain some understanding of the physical proximity and interaction of binding of these subunit species with respect to that of the beta subunit. Possible regulation of the number of receptors for the beta subunit by the other subunits will also be assessed. The overall aim is to obtain knowledge concerning the relationship of quaternary structure of the 7S NGF to target tissue binding and thus biological activity.

105. INDUCTION OF MITOGENESIS BY EPIDERMAL GROWTH FACTOR

Das, M., Dixit, S. N., University of Pennsylvania, School of Medicine, Dept. of Biochemistry & Biophysics, *36th & Hamilton Walk, Philadelphia, Pennsylvania*, 19174, United States of America

The objective of this research is to understand the role of the cell surface receptor in generation of the intracellular activator of DNA replication in epidermal growth factor (EGF) induced stimulation of cellular growth. I have developed a photoaffinity labeling technique for specific covalent radiolabeling of the cell surface receptor for EGF. This procedure has been used for studying the dynamic and migratory behavior of the receptor after binding to EGF. This technique will be further exploited to monitor purification of the receptor. Specific antibodies will be generated against the receptor using the hybridoma technique. The anti-receptor antibody will be used as a tool to test the physiological significance of receptor internalization and processing in the biological action of EGF.

A cytobiochemical assay will be developed for the EGF-induced intracellular activator of DNA replication. Cytoplasmic extracts from EGF-stimulated cells will be introduced into unstimulated cells by microinjection or encapsulation, and the stimulation of DNA synthesis will be measured. Anti-receptor antibody will be used to deplete the assay system of receptors/

processed receptor, and the effects of addition of isolated receptor fragments to these depleted systems will be examined. Properties of the EGF-induced intracellular messenger will be compared with those of the cytoplasmic activator of DNA synthesis in tumor or cancer cells. The cryptic or masked mitogen receptor/processed receptors in transformed cells will be characterized biochemically using anti-receptor antibody. These detailed cytobiochemical studies will aid greatly in achieving my long-term goals: complete definition of the molecular events leading to DNA replication in mitogenic hormone action, and assessment of the roles of mitogenic hormones and their receptors in normal growth and development.

106. EPIDERMAL GROWTH FACTOR RECEPTOR - STRUCT/FUNCT/HOMOLOGY

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B cell hybridomas producing antibodies which bind to a human epidermoid carcinoma (A431 cells) have been obtained and their antibodies partially characterized. These monoclonal antibodies elicit a number of binding specificities. Three of the antibodies elicit specificity for what appear to be the EGF receptors on A431 cells; two others elicit specificity for epitopes which may reside on protein(s) complexed with EGF receptors on the A431 cell surface. Immuneprecipitation studies are in progress to describe the precise cell surface macromolecules which interact with these monoclonal antibodies, and studies of their cross specificity, if any, with other A431 cellular proteins and with murine proteins are in progress. The five antibodies cited above do not alter EGF binding to cells directly, but some do alter EGF binding when a secondary antibody specific for the monoclonal antibody light and heavy chains is added. Tests will be undertaken to screen for possible direct or indirect physiological effects of these antibodies on the regulatory processes normally controlled by EGF.

The antibodies described above are all selected for binding to the extracellular surface of A431 cells. A selection is currently in progress to screen for monoclonal antibodies which interact with intracellular antigenic sites, specifically those which lead to EGF receptor immuneprecipitation.

107. RECEPTOR MECHANISMS IN EPIDERMAL GROWTH FACTOR INDUCED MITOGENESIS

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Our studies on EGF receptors in the A431 vulva epidermoid carcinoma cell line point to the existence of two classes of receptors in nonionic detergent extracts: receptors of one class are highly aggregated and refractory to EGF-induced phosphorylation by ATP, and those of the second are relatively disaggregated and acceptors in EGF-induced phosphorylation. This property will be used as the basis for an assay for receptor clustering in purified membranes in vitro to test for cytoplasmic macromolecules playing essential roles in EGF induced EGF receptor clustering. The high level of aggregation of EGF receptors in membranes has created difficulties in purication of EGF receptors in reasonable yield. We will screen for temperature sensitive mutants blocked in receptor clustering to obviate this difficulty. Characterization of these mutants may also reveal additional information on macromolecules playing roles in EGF-induced receptor clustering and in biological processing which require clustering of EGF and/or its receptors for their induction. The question exists as to whether EGF receptors do or do not serve to transfer phosphate from ATP to other bioactive macromolecules in response to EGF. This concept will be tested in detergent 'permeabilized' EGF responsive cells. The A431 human vulva epidermoid cell line displays hypernormal levels of EGF receptors, a trait which may have endowed these cells with their failure to mature normally, thus giving rise to the tumor of origin of the A431 line. A series of invasive and noninvasive (Bowen's) intraepithelial vulvar tumor cell lines will be established to test for the frequency of this and other unique aspects of the A431 phenotype in vulvar carcinomas.

108. INHIBITION OF EPIDERMAL GROWTH FACTOR BINDING TO SURFACE RECEPTORS BY TUMOR PROMOTORS

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12-0-tetradecanoyl-phorbol-13-acetate (TPA), a potent tumor promotor in carcinogen-initiated mouse skin, causes a marked (about 10-fold) decrease in the affinity of epidermal growth factor (EGF) receptors for (125)-EGF.

In contrast, three compounds structurally related to TPA, but with much reduced biological activtity, did not inhibit (125)-EGF binding. A number of experiments indicates that the mechanisms of inhibition of EGF binding by TPA and native EGF are not identical, and suggests that TPA does not bind directly to EGF receptors. EGF inhibits (125 I)-EGF binding measured at 37 degrees C or 0 degrees C whereas TPA is inhibitory at 37 degrees C but not at 0 degrees C. The inhibitory effect of EGF is rapidly reversed when EGF is removed from the binding medium, whereas the effect of TPA persists after its removal. Exposure of cells to EGF for several hours reduces the number of EGF binding sites on the plasma membrane. The 'down-regulation' is due to endocytic internalization of occupied EGF receptors. We found that TPA, in contrast to EGF, does not induce 'down-regulation' of EGF receptors.

The conclusion that TPA and EGF interact with different binding sites is further suported by experiments in which these agents are shown to trigger biological responses like glycolysis and DNA synthesis in a synergistic manner.

Taken together, these findings suggest that TPA initally binds to sites that are separate from EGF receptors and the TPA-occupied sites subsequently interact with the EGF receptor, thereby reducing their affinity for EGF in a temperature-sensitive fashion.

109. EPIDERMAL GROWTH FACTOR RECEPTOR METABOLISM

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The objective of the proposed research is to study the metabolism of peptide hormone receptors, specifically, epidermal growth factor receptor. Studies will include kinetic analysis of the biosynthesis and degradation of receptor, determination of the pathways involved in these processes, and elucidation of control mechanism regulating these processes.

110. STUDIES ON GROWTH CONTROL EXERTED BY THE PROTEIN NERVE GROWTH FACTOR ON PHEOCHROMOCYTOMA CELLS (CLONE PC12)

Calissano, P., Alema, S., Grasso, A., Mercanti, D., Consiglio Nazionale delle Ricerche, Lab. of Cell Biology, *Rome, Italy*

OBJECTIVE: Understanding the molecular mechanism(s) by which the protein nerve growth factor (NGF) blocks division and induces differentiation in a neuronal population of rat pheochromocytoma (clone PC12) cells.

APPROACH: Characterization of the interaction between NGF and PC12 cells (receptor); internalization and intracellular fate of NGF; identification of cytoplasmic structural components where NGF is located (nucleus, contractile proteins, etc.); molecular changes occurring on the cytoplasmic membrane during NGF-induced arrest of mitosis and neurite growth.

PROGRESS: There are specific receptors for NGF in PC12 cells having Kd of 2 to 10 to the minus 10th power M; after binding NGF is internalized and concentrates around the nucleus; internalization of NGF is influenced by cell density; with confluent cells internalization is blocked; half-life of NGF in PC12 cells is 18-20 hours; NGF stimulates synthesis of new receptors by 6-8 fold within 3 days.

111. RECEPTORS FOR EPIDERMAL GROWTH FACTOR IN TUMOR CELLS

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Studies are to be conducted to explore the mechanisms by which cell proliferation is controlled by a specific mitogenic

agent - epidermal growth factor (EGF). Attempts will be made to isolate and define the biochemical properties of membrane molecules mediating the cellular response to EGF. These molecules include the EGF receptor, the EGF sensitive protein kinase, and the endogenous substrates for the EGF enhanced phosphorylation of proteins.

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112. REGULATION OF MITOGEN ACTIVITY - RECEPTORS FOR EPIDERMAL GROWTH FACTOR

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Important interactions between cells and extracellular signals that regulate cell proliferation/differentiation are critically dependent on the concentration and activity of receptors in target cells. Using epidermal growth factor (EGF) as an example of such an extracellular signal, we will investigate cellular mechanisms which control the metabolism of EGF receptors and influence the interaction of hormone-receptor complexes with the expression of nuclear information.

One of our studies will be to investigate the metabolism, i.e., degradation and synthesis of plasma membrane receptors for EGF and attempt to define cellular and environmental factors controlling these processes. To investigate receptor degradation, protein synthesis will be inhibited by the addition of histidinol, which reversibly blocks the formation of histidinyl-tRNA, to cells and at various times 125I-EGF binding activity will be determined. This system will be used also to measure the synthesis of EGF receptors that occurs when histidine is added to cells pretreated with histidinol. The degradation and synthesis of individual membrane proteins will be analyzed in cells labeled with 125I by lactoperoxidase, 3H-fucose or galactose oxidase-NaB3H4.

Additional studies will investigate the loss of EGF receptors in vitro by sarcoma transformation and in vivo by skin malignancies. Receptor activity will be assayed in heterokaryons formed from transformed cells that have lost EGF receptors and cells that have receptors. Clinically categorized skin cell lines and skin biopsies will be assayed to determine whether the loss of EGF receptor activity correlates with a defined clinical state. Tissue to be analyzed will be representative of normal skin and premalignant, malignant, or hyperproliferative non-malignant skin diseases.

Nuclear or cytoplasmic controls of the sensitivity of gene expression to EGF will be explored in heterokaryons from EGF responsive and non-responsive parents. The nuclei of responsive cells will be inactivated prior to fusion; therefore, heterokaryons will contain EGF receptors from a responsive cell type and a viable nucleus from a non-responsive cell type. The ability of EGF to enhance DNA synthesis in the nuclei of heterokaryons will be assessed by 3H-thymidine incorporation and autoradiography. (Text Abridged.)

113. INVESTIGATION OF THE ROLE OF CARBOHYDRATE IN THE BINDING OF EPIDERMAL GROWTH FACTOR TO ITS RECEPTOR

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The proposed study will examine the involvement of carbohydrate in cell surface receptor-ligand interactions. The receptor for epidermal growth factor (EGF) in normal cells will be compared to the receptor in a mutant cell which has defective glycosylation and decreased EGF binding.

The proposed experiments are designed to answer the following questions: (1) Is carbohydrate directly involved in EGF binding to the receptor? The answer may be determined by measuring the inhibition of EGF binding to cells incubated in the presence of monosaccharides or purified glycopeptides, or by selective removal of specific terminal sugars. (2) Is the observed decrease in the binding of EGF to the mutant cell due to a decreased number of receptors at the cell surface, or to a decreased number of completely glycosylated receptors? To answer this, it is proposed that the receptor can be isolated by affinity chromatography and an antiserum prepared against the purified protein. This antiserum can be used to immunoprecipitate the receptors from the normal and mutant cells. The results of this comparison may determine whether carbohydrate is required for the receptor to become located in the plasma membrane. If carbohydrate is involved directly in the binding of EGF or indirectly by orienting the receptor properly in the membrane, it may be possible to repair the defect in EGF binding to the mutant by addition of the missing sugars to the receptor at the cell surface.

114. THE BINDING OF PLASMA MEMBRANE THAT CONTAINS LECTIN RECEPTORS TO THE ACTIN CYTOSKELETON

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Plasma membrane ghosts have been prepared for axenically grown amoebae of Dictyostelium discoideum by stabilizing the membrane with purified lectin and lysing the cell with Triton X-100. The stable membrane ghosts retain certain distinctive plasma membrane enzyme markers and do not appear to be contaminated with nucleic acid or cytoplasmic components. Regions of membrane with capped lectin can also be prepared by the same procedure resulting in the isolation of caps.

In the proposed study, ghosts and isolated caps will be purified and used as model membranes to study the ultrastructure and biochemistry of the actin cytoskeleton that is associated with plasma membrane containing lectin receptors. The membrane isolation techniques perfected with D. discoideum will be applied to mouse 3T3 cells and their simian virus 40 transformant SV101 in order to investigate changes in the plasma membrane actin interaction resulting from transformation to the neoplastic state.

Peripheral proteins will be extracted from the membranes using mild aequeous buffers. These proteins will be purified and characterized and their properties compared to proteins composing motile cytoplasmic extracts prepared from Dictyostelium amoebae and SV101 cells. Mild extraction with aequeous buffers will remove peripheral proteins while integral components remain associated with the membrane. Inside out and right side out sealed vesicles will be prepared from the extracted membranes and used to study the primary actin binding site on the membrane by a detailed binding study using radiolabelled actin. Nearest neighbor analysis using bifunctional reagents will be used to identify components on the vesicle membrane to which actin binds.

115. FUCOSE BINDING LECTIN - A PMN MARKER AND CHEMOTAXIN

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Fucose binding lectin from Lotus tetragonolubus seeds binds to human granulocytes and stimulates a chemotactic response. Analysis of binding by flow cytometry shows that this lectin binds avidly to neutrophils, quantitatively less to monocytes, and not at all to lymphocytes. Studies will be conducted to determine the nature of the cell receptor and how binding to the receptor effects neutrophil and monocyte function. Additional studies using I125 labeled FBL will be performed to determine the number and heterogeneity of FBL receptors on human myeloid cells. This lectin will also be used as a marker to analyze human leukemias to determine if it will differentiate myeloid and lymphoid leukemias as well as granulocytes and myelocytic leukemias.

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116. LYMPHOCYTE SURFACE GLYCOCONJUGATES

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It is now evident that cell surface glycoconjugates play a central role in mediating the social responses of cells. We propose to determine the structure of the carbohydrate moieties of the murine histocompatibility H-2K and H-2D antigens and relate this to the serotype of these glycoproteins. We further propose to explore the interrelationships that may exist between the processes resulting in the stimulation of mitosis and production of migration inhibition factor (MIF), lymphotoxin, and chemotactic factor by perturbation of lymphocyte surface glycoconjugates.

Employing sensitive and novel serological, immunochemical and biochemical techniques we will perform partial sequence analysis of both membrane bound and purified H-2K and D antigens to assess the possibility of the influence of primary structure variation of the carbohydrate moiety on the physiologic function of the antigens. These studies will also, by comparing the primary structure of the carbohydrate chains and serotype of various H-2 glycoproteins, assess the possibility of a mechanism which controls and/or links the processes responsible for the biosynthesis of the carbohydrate and polypeptide portions of glycoproteins.

Additional studies will be performed to identify molecular species responsible for MIF, LT and ChF activities. Employing a panel of lectins with different lymphocyte receptor specificities, we propose to dissect the individual pathways of lectin induced MIF, LT and ChF by monitoring the appearance of specific molecular species and activities characteristic of these lymphokines.

117. GLYCOPROTEINS OF LEUKEMIC CELLS

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OBJECTIVE: Structural and functional characterization of the glycoproteins of leukemic cells. Studies of Ia glycoproteins are in progress.

APPROACH: Isolation of Ia glycoproteins from B-CLL cells by taurocholate solubilization, Lens culinaris chromatography and/or hydrophobic affinity chromatography, gel filtration and monoclonal antibody affinity purification. Amounts of purified glycoproteins sufficient for peptide map analysis, partial amino acid sequence and glycopeptide sequence should be obtained. Purified molecules will also be reconstituted in protein micelles for testing in immunological assays.

PROGRESS: Five hybridoma lines producing monoclonal antibodies to human Ia antigens have been established; they are directed against various monomorphic determinants of the molecules. They will be purified and used as immunoadsorbents.

118. GLYCOPROTEINS OF LEUKEMIC CELLS WITH PARTICULAR EMPHASIS ON IA GLYCOPROTEINS

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The present research proposal is concerned with the study, at the molecular level, of the glycoproteins of cell surfaces implicated in the control of immune responses. The main objective of the project is the purification of functionally intact human Ia glycoproteins. The latter, also known as DR antigens, are coded for by the D region of the HLA major histocompatibility gene complex. Human Ia glycoproteins, by analogy with mouse Ia glycoproteins, are presumed to play an essential role in 'molecular immune interactions' which take place in the immunological responses to antigens.

The source of material is chronic lymphocytic leukemia (CLL) of the B-phenotype which offers an abundant source of 'monoclonal Ia glycoproteins'. Our immediate goals include: a) Purification of Ia antigens from CLL cells optimizing yield and using non-denaturing conditions in order to isolate functionally intact molecules; monitoring of Ia antigens by cellular radioimmunoassay (CRIA) using cross-reacting mouse allosera and anti-human Ia hybridoma reagents; b) reconstitution of Ia glycoproteins in protein micelles or liposomes for functional assays; c) structural studies of Ia glycoproteins; d) isolation of glycoproteins other than Ia from human leukemic cells.

Previous studies have established that the murine alloserum A.TH anti-A.TL can bind strongly to human CLL cells of B-phenotype. The antiserum is reactive with all patients tested so far. The results suggest that A.TH anti-A.TL serum contains some antibodies reactive with non-allelic Ia determinants, shared between species. The antigenic determinants shared between human and mouse Ia glycoproteins are ascribed to the murine I-E/C subregions.

119. ASSAY OF GLUCOCORTICOID RECEPTORS IN LYMPHOCYTES BY ISOELECTRIC FOCUSING

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OBJECTIVE: To develop a method for the determination of glucocorticoid receptors in human lymphocytes and determine receptors in the blood cells of patients with chronic lymphatic leukemia. Receptor status will be correlated with therapeutic response obtained by different treatment approaches.

APOROACH: Analytical isoelectric focusing in polyacrylamide gels combined with measurement of radioactivity of labelled steroids will be used for quantitative analysis of glucocorticoid receptors.

PROGRESS: Thus far experiments using rat thymus cells have been conducted. Some preliminary results have also been obtained when normal human lymphocytes were used. As an alternative method with which the results are correlated competitive radioisotope assay employed in a conventional way is used. The isoelectric focusing method and its advantages and drawbacks in the analysis of glucocorticoid receptors will be investigated as a first part of the study conducted during the winter of 1979-80 and material from 30 patients with chronic lymphatic leukemia will thereafter be analyzed during the last part of 1980 (collaboration with Docent A. Rajamaki, M.D., Department of Clinical Haematology, University Hospital, and with representatives of the Clinic of Internal Medicine).

Receptor status and clinical response to treatment will be correlated.

120. MYELOID CELL SURFACE RECEPTORS - NORMAL AND LEUKEMIC

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Our previous research established the pattern of acquisition of cell surface receptors during normal human myeloid differentiation. These membrane markers, now being used to examine leukemic myeloid differentiation are detected on cells from only a minority of patients. The objectives of this proposed research are: (1) To induce differentiation in freshly isolated human acute nonlymphoid leukemic cells (ANLL) by incubation with known inducers of differentiation and examine cells for differentiation, viability and recovery; (2) to assess differentiation by cell surface markers for IgG and complement, morphology, and cytochemistry; (3) to investigate whether 5'-nucleotidase and microviscosity can be used as new markers of myeloid membrane maturation; (4) to induce membrane differentiation using HL-60 cells (a human acute promyelocytic leukemia cell line) and clone these cells to examine the mechanism of induced membrane marker expression; (5) to investigate whether chemotherapeutic drugs also will induce differentiation in both HL-60 and freshly isolated leukemic cells; (6) to compare in vitro induction of differentiation to in vivo effects using a diffusion chamber technique in mice; and (7) to compare in vitro chemotherapeutic results with in vivo clinical response in patients receiving the same agents. The overall objective of this research is to provide a useful in vitro model to aid in the diagnosis, treatment, and prognosis of ANLL.

121. RECEPTORS FOR IMMUNOGLOBULIN ON HUMAN LYMPHOMA-LEUKEMIA

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We propose to study B cell differentiation pathways with respect to Ig receptors and SmIg in order to (1) characterize B cell differentiation, (2) delineate clinically important subgroups of patients with lymphoid malignancies who have differing

prognoses based on marker phenotype. In order to do so, we will utilize 2 model systems. The first is a normal neonatal mouse spleen model which will provide information regarding the ontogeny of B lymphocyte receptors during fetal development. The second is the study of human lymphoid neoplasms. Fluorescent labelled reagents will be utilized and cells viewed under fluorescence microscopy. Cells will also be analyzed and sorted by the fluorescent activated cell sorter (FACS IV), particularly double fluorescent labelled cells. Heavy chain specific EA (Ig) complexes will be used to compare to the results obtained with Ig aggregates. Clinical information derived from patients with neoplasms studied for Ig receptors will be analyzed to determine whether there are differences in survival, response to treatment or other clinical parameters which correspond to differing phenotypic profiles.

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122. THYROID HORMONE RECEPTORS IN LEUKEMIC CELLS

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Thyroid hormone receptors have been demonstrated in circulating lymphocytes by three research groups including ours (K. Liewendahl, S. Rosengard and B.-A. Lamberg.: Nuclear binding of triiodothyronine and thyroxine in lymphocytes from subjects with hyperthyroidism, hypothyroidism and peripheral resistance to thyroid hormones. Clin. Chim. Aceta 83:41-48, 1978). The objective of this new project is to characterize thyroid hormone receptors in leukemic lymphocytic cells and later also in leukemic myeloid cells. The demonstration of high-affinity thyroid hormone receptors might have therapeutic implications. One may assume that the administration of large amounts of metabolically inactive thyroid hormone analogs with affinity for such receptors could affect leukemic cells more deleteriously than healthy cells due to the marked difference in the metabolic rate. Previous studies have shown that the metabolic activities of the white cells are partly governed by the thyroid hormones.

123. MODULATION OF CELL SURFACE RECEPTORS FOR THE MAJOR ENVELOPE GLYCOPROTEIN OF MURINE LEUKEMIA VIRUS

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The modulation of murine thymocyte surface receptors for the 71,000 dalton major envelope glycoprotein, gp71, of Rauscher murine leukemia virus has been examined utilizing a highly specific (125I) radiolabeled gp71 binding assay. Cell surface receptors for ecotropic murine leukemia virus respond similarly to ligand-induced topographical modulation as do other cell surface receptors indicating that their anchorage and movement in the cell membrane is mediated via microfilament and microtubule cytoskeletal structures.

A reduction in the affinity constant, Ka, and number of available receptor sites on thymocytes for gp71 by adenosine triphosphate has been demonstrated. This effect is specific in that no modulation is seen with the other ribonucleotides, deoxynucleotides or cAMP, ADP or AMP. The inclusion of an ATP generating system in the receptor assay reduced the amount of ATP needed to reduce gp71 binding. Non-hydrolyzable analogs of ATP, i.e. App(NH)p, also modulated receptor activity suggesting an allosteric interaction of ATP with the gp71 receptor complex.

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124. STUDIES ON GLUCOCORTICOID RECEPTOR PROTEINS AND THE EFFECTS OF GLUCOCORTICOIDS ON LEUKOCYTES AND LEUKEMIA CELLS

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OBJECTIVE: Development of assay methods for determination of glucocorticoid receptors and mechanisms of action of glucocorticoids on DNA-, RNA-, and proteins synthesis in human normal leukocytes and leukemia cells. This is intended to aid as a predictive test for selection of patients likely to respond to glucocorticoid therapy.

APPROACH: After cell isolation and cytosol preparation, the glucocorticoid receptor is labelled with 3H-glucocorticoid. The steroid-receptor complex is subjected to limited proteolysis. The resulting merorecptor which still binds the ligand is then separated by isoelectric focusing in polyacrylamide gel. The translocation of the labelled cytosolic glucocorticoid receptor to the nuclei of leukemic cells will also be studied. These data will be correlated with the clinical and hematological data on the patients.

PROGRESS: Similar isoelectric focusing characteristics of the glucocorticoid receptor were found in human normal lymphocytes, human leukemia cells and rat hepatocytes. Consecutive patients with different recently onset leukemias have all shown detectable glucocorticoid receptors with broad concentration ranges within each disease. The anti-rat glucocorticoid receptor antibody gives promising results in preliminary assays and could prove to be valuable in studies on the structure and function of the human glucocorticoid receptor in normal lymphocytes and leukemia cells.

125. BIOLOGICAL AND CLINICAL SIGNIFICANCE OF GLUCOCORTICOID RECEPTORS IN HUMAN LEUKEMIA

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A comparative study of glucocorticoid receptor (GR) levels in whole cell suspensions vs. cytosol preparations has been done in 41 cases of human leukemia (12 CLL, 15 ALL and 14 ANLL). The results indicate that the two methods of analysis furnish inter-correlatable values, independent of the type of leukemia. Therefore, in the future this will enable GR determination studies even in cases from distant centers by using frozen cells.

In 19 cases of childhood ALL and 15 cases of adult ALL the quantity of GR in leukemic lymphoblasts correlates positively with the induction of complete remission. A follow-up study is in progress to determine if the length of remission and survival of these patients also correlates with receptor levels.

The in vitro corticosensibility of ALL cells (in terms of metabolic inhibition) does not seem to provide any indication of the patient's responsiveness to therapy. The concentration of GR, independent of their functional activity, seems to be an expression of the degree of cell maturation (results obtained from studies on human thymus are now being done on leukemic cells).

A micromethod for the measurement of the enzyme glutamine synthetase in leukemic cells has been established. The purpose is to develop a specific test of the receptor's functional activity in cells exposed to glucocorticoids.

126. GLUCOCORTICOID RECEPTORS AND HORMONE RESPONSE OF LYMPHOMA CELLS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

127. STUDIES OF LEUKOCYTE AND PLATELET MEMBRANE RECEPTORS

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The project will focus on the structure and function of leukocyte membrane receptors and surface antigens. The project has four interrelated long-term objectives. The first of these is to characterize normal leukocyte membrane surface markers for their distribution on different leukocyte types and for their order of appearance during leukocyte maturation. The project will not be restricted to one leukocyte type, but will encompass the spectrum of bone marrow nucleated cells, including erythroid precursors and megakaryocytes. The findings on normal leukocytes form the basis for the second objective of the project, to find and characterize leukemia specific membrane markers and determine which of these may have prognostic significance for early determination of important clinical subtypes or stages of leukemic disease. The third goal is to analyze lymphocyte membranes by scanning electron microscopy and determine the distribution and association of different types of receptors on individual cells. The final goal of the project is to study platelet membrane receptors for IgG, Fc (Fc receptors) and determine the relationship of these receptors to platelet receptors for clotting factor VIII. Preliminary studies have suggested that the Fc receptor may be either the same entity as the factor VIII receptor or located adjacent to it. Since IgG containing immune complexes have been shown to block factor VIII receptor activity, platelets from patients with immune complex-associated disease will be examined for platelet dysfunctions caused by a common blockade of Fc and factor VIII receptors.

128. GLUCOCORTICOID BINDING IN HUMAN LEUKEMIA

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The objective of this proposal is to compare the physicochemical properties of cytoplasmic and nuclear glucocorticoid binding components of a variety of corticoid-sensitive and corticoid-resistant human leukemic lymphocytes, and to study the mode of glucocorticoid-receptor complex interaction with the nucleus in these cells. Hormone-receptor complexes will be characterized by gel filtration, sucrose density gradient centrifugation, isoelectric focusing and ion exchange chromatography. The information we will obtain will help us understand the role of receptor in the mechanism of glucocorticoid action on corticoid-sensitive leukemic lymphocytes and will help to clarify the basis for resistance in receptor (positive), nuclear transfer (positive) lymphocytes that fail to respond to steroid therapy.

129. HISTAMINE H2 RECEPTOR T-SUPPRESSOR CELLS IN CANCER

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Suppressor T-cells play an important role in the biology of human tumors. The focus of the work we propose is the suppressor T-cell subset which is characterized by the possession of a surface receptor for histamine of the H2 type (H2R plus cells). Previously we have studied this cell from biochemical and immunological perspectives. This has included the solubilization and partial purification of the specific histamine H2 receptor as well as the description of the histamine activation of the cell to a functional suppressor lymphocyte. Our work is a detailed study of the relationship of this specific suppressor cell to malignant disease. In order to do so we will characterize and quantitate histamine receptors on the T-cells of patients and animals with malignant disease; determine the effect of histamine and its antagonists on the functional immunological activity of the H2R plus cell; determine if histamine and its antagonists have a role in the therapy of cancer; and determine the role that serum histamine has in the pathophysiology of malignancies.

130. T-CELL RECEPTOR AND EFFECTOR MOLECULES

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Defining the T cell receptor has been one of the most persistent problems of modern immunology. Although several studies have shown that T cells express idiotypes similar to those on immunoglobulin (Ig), many other investigations have been unable to document other Ig-associated determinants on T cells. It has been proposed that T cells utilize receptors containing Ig heavy chain variable (Ig VH) domains with different constant regions. The rabbit is a useful species for such studies because it possesses a well-characterized, unique set of Ig VH allotypes that are framework markers for all known rabbit Ig classes. The major obstacle to doing such experiments has been the difficulty in obtaining highly purified functional rabbit T cells. During the past year, this laboratory has developed several continuously proliferating rabbit T cell lines, some of which are functionally active as cytotoxic cells. These cells will be used in experiments with a variety of anti-Ig reagents, including antisera to the Ig VH framework determinants encoded by the a locus allotypes. The results of these studies should confirm or refute the model of the T cell receptor containing an Ig VH domain.

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131. BIOCHEMICAL AND IMMUNOLOGICAL STUDIES OF MEMBRANE PROTEINS INVOLVED IN CELLULAR IMMUNITY - T-CELL RECEPTOR

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There are two major lines of lymphoid cells for distinct immunological functions: the thymus-derived T cells for cell-mediated immunity and the bursa-derived B cells for humoral immunity. Separation of these two cell lines can be easily accomplished in the chicken by in ovo bursectomy.

The objective of the proposed research is to biochemically and functionally characterize macromolecules of the T-cell surface involved in recognizing antigen (T-cell receptors) and to study the molecular events in the interaction between T and B cells. For this objective, the following experiments will be performed: (1) isolation of antigen-specific suppressor T cells; (2) demonstration of antigen-binding cells by autoradiography; (3) enrichment of antigen-binding cells; (4) identification of T-cell receptors from the enriched antigen-binding cells; (5) preparation of anti-receptor sera; and (6) further biochemical analysis (i.e., immunochemical characterization and peptide structures) of T-cell receptors vs B-cell Ig receptors.

132. TRIIODOTHYRONINE RECEPTORS AND THYROTROPH NEOPLASIA

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The molecular events leading to pituitary thyrotroph cell division with eventual neoplastic transition will be studied in rats and mice during acute and chronic hypothyroidism. The decrease in L-triiodothyronine (T3)- concentration in hypothyroidism results in the desaturation of the nuclear T3 receptor which is a chromatin nonhistone protein and causes an increase in cell function (TSH secretion) and rate of cell division. Since this class of chromatin proteins plays a critical role in activation of the genome in all phases of the cell cycle, it is likely that changes in these nuclear proteins during hypothyroidism lead to an altered thyrotroph cell cycle resulting in hyperplasia and neoplasia.

The proposed studies will first explore the cell cycle of the normal thyrotroph, the distribution of the nuclear T3 receptors among the different cell lines in the anterior pituitary, the distribution and metabolism of T3 in the mouse, and the time of onset of thyrotroph cell division in the rat. The baseline information obtained from these studies will then be used to determine: a) alterations in the physicochemical characteristics of the nuclear T3 receptor; b) the regulation of TSH secretion by T3 and

thyrotropin releasing hormone (TRH); and c) nonhistone chromatin protein patterns, synthesis, turnover, and phosphorylation in different stages of thyrotroph transition from normal cells to undifferentiated metastasizing neoplasms. Parameters a), b), and c) will be studied in normal thyrotrophs, at the onset of thyrotroph hyperplasia, during hyperplasia, in thyrotrophic pituitary tumors, in transplanted hormone-dependent thyrotrophic tumors, and in autonomous hormone-independent thyrotrophic tumors. These studies should lead to a well-characterized model system which should provide new insights into the interrelationships between the chromatin protein T3 receptors, cell function, chromatin protein metabolism, and cell replication.

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133. TRIIODOTHYRONINE RECEPTORS AND NONTHYROIDAL DISEASES

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The mechanism whereby euthyroidism appears to be maintained in patients with nonthyroidal disease (NTD) who have decreased plasma total and free triiodothyronine (T3) concentrations (low T3 syndrome) will be studied in patients with neoplastic diseases and in rats bearing the Walker 256 carcinoma in the thigh. Similarly to humans with NTD, tumor-bearing rats have a decrease in plasma T3 but their plasma thyrotropin (TSH) concentration is normal. In the rat, plasma thyroxine (T4) concentration is decreased as well. The observation that hepatic nuclear T3 receptor concentration is decreased in the tumor-bearing rat will be extended to an examination of receptor concentration in rat anterior pituitary and human lymphocytes. A decrease in lymphocyte T3 receptor concentration in patients with NTD and the low T3 syndrome will indicate that the tumor-bearing rat may be a useful model for studies of receptor regulation, nonhistone protein metabolism and biologic responses to T3 in NTD. The effect of neoplasms on biologic responses to thyroid hormones will be determined by measurement of the augmentation of plasma TSH concentration in response to a further decrease in plasma thyroid hormone, the release of TSH after administration of thyrotropin releasing hormone (TRH), and increase in thyroid hormone sensitive hepatic malic enzyme and alpha-glycerophsophate dehydrogenase activities.

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134. TSH RECEPTOR(S) IN HUMAN THYROID CARCINOMAS

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The presence of thyrotropin receptor(s) in thyroid tumor, relevant to a correct post-surgical therapy, may be investigated by means of a systematic study of the adenylate cyclase response to TSH. Thus TSH-dependent cyclic AMP accumulation in randomized thyroid slices, obtained from tumorous tissue, offers a good index of TSH receptors in tumors.

Basal cAMP levels are generally more elevated in tumorous tissue than in normal tissue, while the response to TSH is reduced or absent. However, an aspecific stimulus of the adenylate cyclase as prostaglandin E2 at elevated concentration (0.1 mM) produces a normal response in differentiated carcinomas.

To further elucidate these abnormalities of adenylate cyclase-cyclic AMP system of thyroid tumors, studies on cAMP-dependent protein kinase and phosphodiesterase activities are in progress. Furthermore, thyroid carcinomatous cell cultures are established and 3H-leucine as iodide incorporation in thyroglobulin, 3H-thymidine uptake and TSH cell surface receptors are studied.

135. MOLECULAR CHARACTERIZATION OF THE EGF RECEPTOR FROM THE A431 HUMAN CARCINOMA

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Addition of epidermal growth factor (EGF) to cultured, responsive cells lead first to the internalization of the EGF receptor and then to cell cycle events which move the cell from G1 into the S phase (DNA synthesis). These two separate processes have the same dose response to EGF, and conditions which favor a continuous delivery of receptor into the cell during the requisite 6-8 hr induction period are optimal for induction of DNA synthesis. The sponsor's laboratory has identified the EGF receptor of a human carcinoma cell line as a specific 170,000 dalton macromolecule and is currently engaged in the purification of this EGF receptor. If that purification has not been completed upon my arrival there, I will participate in that project. Once purified receptor is at hand, I will use it to generate monospecific antibodies to receptor. These in turn will be used to determine the disposition of the receptor in the membrane. Dr. Fox and his colleagues have shown that the receptor has the properties of an integral membrane protein, but they have no information on whether the receptor protein does or does not span the membrane. The answer to this question is crucial to the formulation of molecular models of how mitogenic hormones such as EGF work. Once an antibody preparation is at hand, cells carrying the EGF receptor will be used to adsorb out those species of anti-EGF receptor Ig which interact specifically with the protein of the EGF receptor that is displayed outside the cell. The remaining nonadsorbed anti-receptor Ig should be specific for that portion of receptor which is a) exposed to the cytosol face of the membrane or b) buried in the hydrocarbon core of the membrane. This antibody preparation will be used to 1) test for a portion of receptor which is exposed to the cytosol membrane face, 2) prepare ferritin antibody to that portion of receptor to test for its disposition on the membrane in ultrastructural experiments and 3) test for the fate of the putative cytosol exposed receptor fragments in cells stimulated by the addition of EGF. In anology to the estrogen receptor protein, does a fragment of the EGF receptor become transported to the nucleus to serve as the second messenger in EGF action?

136. HORMONE AND RECEPTOR REGULATION OF MAMMARY CANCER

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OBJECTIVE: The role of hormones and their receptors in the growth and development of mammary carcinoma under both in vivo and in vitro conditions will be studied. In order to better understand the hormonal dependence of mammary tumors, two experimental models (DMBA and NMU) will be employed and effects on tumor growth and regulation of hormone receptor levels will be studied.

Prolactin receptors will be purified from rabbit mammary glands. This will permit the production of antisera specific to the receptor protein and allow a more accurate and reliable means of measuring prolactin receptors, with the hope of better understanding the role of prolactin in the development of human breast cancer. In addition, this approach will be important to follow the intracellular migration of the prolactin receptor.

APPROACH: Organ cultures of DMBA and NMU-induced rat mammary tumors will be established and the effect of various steroid and peptide hormones as well as cellular and metabolic inhibitors will be investigated for their role in tumor growth. In addition, the regulation of receptors for estradiol, progesterone, dexamethasone as well as of prolactin will be examined and correlated with receptor regulation in normal mammary tissue. The role of prolactin in the growth of NMU-induced mammary tumors will be examined in detail in vivo studies using the prolactin lowering drug CB-154, as well as a

specific antiserum to prolactin and the prolactin receptor. These proposed studies should help clarify the regulatory mechanisms involved in tumor growth as well as possibly offering new approaches to investigate the mechanisms by which prolactin induces its actions in its target tissues. Prolactin receptors will be purified from rabbit mammary glands by two stage affinity chromatography. Conventional and monoclonal antibodies will be produced, and these will be utilized for in vivo and in vitro studies to follow the intracellular fate of prolactin receptors following internalization.

137. CHARACTERIZATION OF DOPAMINERGIC RECEPTOR CHANGES IN NEUROBLASTOMA TUMOR CELLS DURING CYCLIC AMP INDUCED DIFFERENTIATION

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Treatment of neuroblastoma adrenergic cell clones with agents which increase intracellular cyclic AMP level results in morphological 'differentiation'. These changes are accompanied by an increase in the responsiveness of adenylate cyclase to dopamine and norepinephrine stimulation. The objectives of the proposed studies are to characterize the components of adenylate cyclase coupled catecholamine receptors in neuroblastoma cells and to evaluate their role in changes in the responsiveness to catecholamines during induced morphological 'differentiation'. Catecholamine receptors will be directly studied by radioligand binding studies and their pharmacology from binding assay will be compared to the results of adenylate cyclase stimulation. The role of guanine nucleotide on receptor effector coupling will be studied by evaluating their modulatory role on agonist binding to their receptors and on adenylate cyclase stimulation. Guanine nucleotide regulatory protein will be studied by computer modelling of agonist binding, by direct labelling following ADP reboxylation with (32P)-NAD in the presence of cholera toxin and by reconstitution of the AC- mutant of S49 lymphoma cells with soluble extracts from nucleoblastoma cells. Using these methods, changes in the density of receptor or regulatory units which might accompany the increase in responsiveness during 'differentiation' will be assessed. Neuroblastoma cells constitute a model system for studying the molecular mechanism of alteration in the responsiveness of target cells to catecholamines.

138. PROTEIN AND STEROID HORMONES IN PROSTATIC NEOPLASIA

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We are examining the hypothesis that quantitation of total androgen receptor content of primary and/or metastic human prostatic adenocarcinoma may provide a clinically useful prognostic index which would (a) identify patients who would benefit from hormonal therapy and (b) be correlated with clinical course. This prospective study correlates total prostatic adenocarcinoma androgen receptor content with: 1) response to therapy; 2) tumor stage; 3) histopathologic and biochemical characterization of tumor tissue; and 4) clinical course. Adequacy of therapy is monitored by selective evaluation of plasma hormones, plasma enzymes, and standard clinical evaluation. Response is scored by objective and subjective criteria.

139. ENDOCYTOSIS AND METABOLISM OF ASIALOGLYCOPROTEINS BY HEPATOCYTES

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Research outlined in this proposal is intended to address several questions related to receptor-mediated endocytosis of asialoglycoproteins. Firstly, a morphological examination of the receptor for asialoglycoproteins on the surface of lysosomes is described. This portion of the proposed project relates to the possible mechanism by which receptor is spared during ligand catabolism. Secondly, the mechanism by which ligand and receptor are internalized will be investigated using fluorescently labeled antibodies to the receptor as well as fluorescent ligand. Specifically, the possible involvement of coated pits and coated vesicles in this process will be studied. Lastly, the possible movement of receptor from the plasma membrane to the cell interior and its return for reutilization will be addressed with an immuno-isolation procedure for receptor-ligand complex. This system provides an excellent model system for receptor-mediated protein uptake since both the receptor and the ligands have been purified and partially characterized.

140. REGULATION OF INSULIN AND INSULIN-LIKE GROWTH PEPTIDE ACTION IN HEPATOMA CELLS

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Insulin and multiplication stimulating activity (MSA), an insulin-like growth peptide, induce the enzyme tyrosine aminotransferase and increase the rate of amino acid transport in dexamethasone-treated rat hepatoma cells in tissue culture. Insulin also causes a time-dependent decrease in cellular responsiveness to further insulin action. The objective of this project is to study the mechanisms by which insulin and MSA regulate cellular responsiveness to their own actions. The time course, concentration-dependence, specificity, reversibility, cell-cycle specificity, role of cytoskeletal elements, and the role of macromolecular synthesis in the desensitization phenomenon will be investigated. Both the regulation of plasma membrane receptors for these two hormones and the regulation of cellular responsiveness (transaminase induction and amino acid transport) to these agents will be studied in parallel. Correlation, or dissociation, of these two aspects of hormonal regulation is important for an understanding of the cellular mechanisms of hormone action. An understanding of the regulation of hormone responsiveness may also be relevant to understanding of the pathophysiology of disease states in which hormone resistance is a part. Finally, these studies should provide information on the cellular actions of insulin-like growth peptides.

141. CELL SECRETION AND MEMBRANE FORMATION IN THE PANCREAS

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Studies in our laboratory will focus on four areas in the coming year. First, we propose to synthesize a radioactively labeled (125I) photoaffinity labeling analog of CCK-OP for use in defining the biochemical properties of the CCK receptor in the adult acinar cell and for use as a probe in determining appearance of CCK receptors in the developing pancreas. Second, we will examine, using biosynthetic labeling protocols and SDS-PAGE, the pattern of appearance of cell surface glycoproteins during embryonic pancreatic histogenesis. Additional studies using immunocytochemical approaches will follow changes in basal lamina and other connective tissue components during gland morphogenesis.

Third, we plan to pursue studies on a differentiated rat acinar cell tumor with the aim of determining the potential role of basal lamina constituents in tissue disorganization in the neoplasm and of defining plasmalemmal components that may be responsible for interactions of cells with the extracellular matrix. Finally, studies will continue in an effort to label in situ the apical plasmalemma of the mature acinar cell with 125I for comparison with the membrane of the zymogen granule which specifically interacts with this plasmalemmal domain during exocytosis. Along the same line, we propose to identify the subcellular localization of proteins that are phosphorylated in the acinar cell during secretagogue stimulation of exocytosis.

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142. LYMPHOPROLIFERATION AND CELL SURFACE RECEPTORS

Pattengale, P. K., University of Southern California, School of Medicine, Dept. of Pathology, *2025 Zonal Ave., Los Angeles, California*, 90033, United States of America

This is part of a broader project. A summary of this subproject is not available.

143. RECEPTOR STUDIES

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This is part of a broader project. A summary of this subproject is not available.

144. REGULATION OF THE CELL CYCLE AND DIFFERENTIATION OF NEUROBLASTOMA CELLS IN VITRO - DYNAMIC PROPERTIES OF PLASMA MEMBRANE RECEPTOR SITES

van Zoelen, E. J., de Laat, S. W., van der Saag, P. T., Rijksuniversiteit te Utrecht, Hubrecht Lab., *Uppsalalaan 8, 3584 CT Utrecht, Netherlands*

OBJECTIVE: To reveal the molecular mechanism underlying the regulation of growth and differentiation of neuroblastoma cells, in particular the possible role of r odulation in the expression of plasma membrane receptor sites.

APPROACH: The intrinsic modulations n the expression of a number of specific plasma membrane rece tor sites is investigated. A possible relationship between the ex ression of receptor sites and their dynamic properties, measured as their lateral and rotational mobilities, is under study, with emphasis on the role of lipid-protein interactions.

145. COMPLEX OLIGOSACCHARIDES IN NEMATODE DEVELOPMENT

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We are studying the role of complex carbohydrates in the development of the nematode Caenorhabditis elegans. These molecules, the glycosphingolipids and glycoproteins of the outer face of cell membranes, have been implicated in cellular processes including division, migration, and adhesion. I intend to characterize the glycoconjugates made by C. elegans and to determine their cellular distribution by fluorescence microscopy of carbohydrate binding proteins, including plant lectins, bacterial toxins, antisera to carbohydrate antigens, and endogenous nematode lectins purified by affinity chromatography on carbohydrate coupled supports. Similarly, we will attempt to localize the endogenous lectins by immunofluorescence or with fluorescent carbohydrate probes. In this way, we hope to understand how sugars and receptors interact during development.

146. RECEPTOR AGGREGATION AS A PRIMARY EVENT IN SIGNAL TRANSDUCTION IN RECEPTOR MEMBRANES

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The following research project is aimed at understanding the mechanism of membrane receptor functioning. It is particularly directed at testing the proposal that the first event which follows receptor activation by substrate binding or light absorption is a cooperative aggregation of receptor monomers leading to the formation of a channel through which a transmitter enters the cell and initiates a cascade of consequent events. We have chosen to specifically study the photoreceptor, rhodopsin, and the acetylcholine receptor since membranes enriched with either of the receptors are relatively easy to obtain. Furthermore, both receptors can be purified and reconstituted into phospholipid vesicles while retaining their functionality. Our approach in this project will be to follow the aggregation states of these receptors in the membrane before and shortly after their activation. In our study we intend to use three independent techniques and both the native and the reconstituted membranes. The techniques will include fluorescence energy transfer, quasi-elastic light scattering and photochemical cross-linking.

147. FUSIGENIC ACTION OF SPERM BINDING WITH CELL MEMBRANES AND ARTIFICIAL LIPID VESICLES

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The sperm protein, bindin, mediates the species-specific adhesion of sperm to sea urchin eggs. Bindin can be isolated in pure form in milligram quantities. Bindin may also be a fusigenic protein mediating the fusion of gamete membranes. We will investigate the association of bindin with natural and artificial membranes to determine if it enhances membrane fusion. We will also determine what parts of the bindin molecule associate with the hydrophobic portion of the membrane and which parts protrude from the membrane. A bindin-receptor glycoprotein has been isolated from the egg surface. Using vesicles constructed with either bindin or its receptor, we will study the interaction of these vesicles with sperm and egg membranes and with each other. Do bindin-vesicles have an affinity for cell membranes? Will they promote vesicle-vesicle or vesicle-membrane fusion? These studies may be basic to the development of a true biochemical understanding of cell-cell recognition and interaction, as well as membrane fusion events.

148. SOMATIC CELL GENETIC STUDY OF MAMMALIAN CELL SURFACE RECEPTORS AND ANTIGENS

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The objective of my current research is to dissect the genetic control of human cell surface functions with emphasis on the receptors for two antagonistic peptide hormones, insulin and glucagon. Attempts are being made to identify the genes responsible for production and regulation of the active receptor molecules and of the regulatory enzymes involved in the corresponding metabolic pathways; to understand the genetic variation in the receptor molecules and their effects on the functions of metabolic enzymes; to assign these genes to specific human chromosomes and localize them to specific regions of the chromosomes; and to understand coordination and/or hierarchy of gene action in the receptor-mediated hormonal signal transfer systems. The binding of a peptide hormone molecule to its specific receptor on the target cell surface is an important step in the regulation of mammalian cellular proliferation. Loss of such hormonal control might result in malignant cell formation.

I have been using somatic cell genetic approaches to achieve these goals in combination with biochemical and immunological techniques. A series of genetically altered mutant cells which are defective in each of the components essential for hormone action and for further specific steps in the signal transfer and in the metabolic sequences controlled will be isolated from a cell population mutagenized in vitro or from mice with identified diseases. Intra- and interspecific somatic cell hybrids will be produced using microcell-mediated chromosome transfer techniques in various combinations of mutant cells and wild-type cells of human and mouse origins.

My research is also directed toward the genetic dissection of human cell surface antigens, using specific antisera produced against highly segregated human x mouse somatic cell hybrids. New methods of assaying human phenotypes on a single cell basis will be developed with the use of specific antisera in combination with fluorescent and histochemical staining. Differences between normal and neoplastic cells in various aspects of cell membrane structure and function will be studied using genetically defined phenotypic markers.

The ultimate goal of my research project is to understand the mechanism of cellular response to external signals, using insulin and glucagon as model systems, and to obtain information on fine map of human genome and control of gene action.

149. MELANOCYTE STIMULATING HORMONE RECEPTOR CHARACTERIZATION

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We plan to continue our investigations on characterizing the melanocyte stimulating hormone (MSH) receptor. We will

systematically synthesize a variety of structural and diastereoisomeric alpha-MSH analogs and evaluate their biological activity in vitro by frog skin assay, melanoma adenylate cyclase, melanoma cyclic AMP, and melanoma tyrosinase assays. We hope to produce melanotropin analogs of high potency that exhibit prolonged binding and are resistant to enzymatic degradation. The peptides will then be radioiodinated and used for melanoma tumor localization. They might also prove useful as drug carriers for melanoma chemotherapy. To further characterize the melanotropin receptor we plan to design a radioreceptor assay and use radioiodinated melanotropins to determine the specificity and structural requirements for melanotropin receptor binding. The relationship between receptor binding and signal transduction leading to adenylate cyclase activation and cyclic AMP production will be determined. Certain analogs may be produced whose receptor binding is uncoupled from signal transduction. These antihormones (antimelanotropins) may also be useful in melanoma tumor localization and as drug carriers without affecting undesirable physiological responses. The enzymology of melanotropin analog degradation will be investigated. Our melanotropin analogs will also be studied for their in vivo effect on integumental melanogenesis in two rodent models: the agouti, viable yellow (Avy) mouse and the Mongollian hamster. Melanotropin effects on a number of behavioral paradigms in the rat will be investigated. These melanotropin analogs may be useful in studies on fetal adrenal function and other putative melanotropin target tissues.

150. ROLE OF IRON IN THE CONTROL OF NORMAL AND MALIGNANT CELL GROWTH

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One of the major problems confronting the molecular biologist is the understanding of the molecular events that control growth of mammalian cells. In general, the multiplication of cultured untransformed mammalian cells requires serum and in defined media (media without serum) it requires the presence of the serum iron carrier glycoprotein transferrin (Tr). Many transformed cells have lower serum requirements than the untransformed counterparts and some which proliferate in defined media do not require Tr. From these considerations, it can be suggested that some transformed cells must be able to regulate iron transport by a mechanism different from Tr mediated iron uptake. Thus, the understanding of differences and similarities in the mechanisms of iron transport between normal and transformed cells may be of great significance for the understanding of transformation. In order to study this problem two major complementary lines of work are in progress: 1) the elucidation of the interaction of Tr with specific receptor, particularly in normal cells, and its relation to cell proliferation; 2) identification of ways in which transformed cells transport iron in the absence of Tr.

151. COMPLEMENT MEMBRANE ATTACK PROTEINS BINDING AND BIOSYNTHESIS

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Complement has been considered to be mediator of humoral immunity active in antibody-mediated lysis of bacteria and cells, and in anaphylactic and Arthus reactions. It has been largely ignored as a potential mediator of cellular-immunity. The discovery that tissues and cells of the reticuloendothelial system syntesize complement proteins and that lymphocytes, monocytes, and polymorphonuclear neutrophils possess cellular membrane receptors for complement proteins, clearly suggests the need for studies to determine whether complement plays a role in cellular-immunity. It is the objective of the present proposal to provide the basic information necessary for such functional analyses.

The applicant has recently reported the finding of neoantigenic-markers for the membrane attack complex of complement on lymphocytes. The complement membrane attack complex is a macromolecular complex C5b, C6, C7, C8 and C9 which induces irreversible membrane changes, osmotic swelling and lysis of cells. The studies outlined in the present proposal will seek to answer three questions: First, are C5, C6, C7, C8 or C9 present on the surface of peripheral blood lymphocytes? Second, does C5, C6, C7, C8 or C9 singly (or in combination) bind to the lymphocyte membrane, i.e. do receptors exist for terminal complement proteins? Third, do lymphocytes synthesize C5, C6, C7, C8 or C9 during culture and is biosynthesis under immunological control. The methods used to answer the questions will include: immunofluorescence microscopy, cell-surface radiolabeling techniques, binding reactions with radiolabeled complement proteins, metabolic radiolabeling techniques, and hemolytic assays. These studies are made possible by the recent development of methods which allow the routine purification of complement proteins and production of monospecific antisera to these proteins.

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152. FC RECEPTOR STRUCTURE AND FUNCTION

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Plasma membranes of macrophages and lymphoid cells, both normal and of tumor origin, bear receptors for IgG (Fc receptors). These receptors are vital to phagocytosis of antibody-coated particles and to antibody-mediated cell-cytotoxicity (ADCC). They are implicated as well in a number of biological processes, including regulation of antibody production, modification of cytotoxic and mitogenic responses, stimulation of suppressor cell function, and mediation of enhancing effects of immune complexes on tumors and allografts. A more precise knowledge of the structure of these receptors is needed.

I am proposing to isolate and characterize Fc receptors on mouse and human macrophages and lymphocytes, both normal and from tumors. Established methods which have been successful in preliminary experiments will be utilized. Techniques for radiolabeling membrane macro-molecules, purifying membranes, lysing membranes with detergent, and purifying receptors by affinity chromatography will be used. I will then characterize the receptors as to molecular weight and composition for purposes of analyzing the suspected heterogeneity of Fc receptors. Antisera against the purified receptors will be prepared both for the purpose of purifying large amounts of solubilized Fc receptor for sequence analysis and to delineate Fc receptor function. For the purpose of studying Fc receptor function, Fab or F(ab')2 antisera specific for Fc receptors will be utilized to block or modulate Fc receptor activity in several in vitro assays of immune function. The results of these studies will allow a more precise understanding of the contribution of Fc receptors to the various physiological and pathological mechanisms outlined above.

153. RECEPTOR DYNAMICS AND NORMAL/TUMOR CELL FUNCTION

Bankert, R. B., Roswell Park Memorial Inst., *666 Elm St., Buffalo, New York*, 14263, United States of America

Several recent hypotheses suggest that cell recognition and growth are regulated in large part by epigenetic alterations in the structure of cell surface receptors as well as by changes in the receptor mobility and distribution. Bone marrow derived lymphocytes (B cells) provide a unique model for studying the regulation of eukaryotic cells since B cells possess membrane associated antigen-binding receptors. Ligands binding to the receptors may initiate either specific activation or specific suppression of cellular proliferation and differentiation. Receptors are present on precursors of antibody-forming cells as well as on immature antibody-forming cells. The receptors appear to be lost through differentiation since they are not detectable on mature antibody-forming cells. I propose to study and compare the dynamics of receptors on a normal and a neoplastic antibody-forming cell pair following the interaction of the receptors with either a stimulator ligand or a suppressor ligand. The cells of the normal and neoplastic cell pair that I will use are identical in at least two respects, i.e., they each synthesize and secrete a structurally identical anti-dextran antibody, and each cell pos-

senses receptors which bind dextran. Dextran B1355 stimulates the proliferation of normal anti-dextran antibody-forming cells while the same dextran, when oxidized, specifically suppresses the normal proliferative response. The oxidized dextran also specifically suppresses the in vivo proliferation of the neoplastic anti-dextran antibody-forming cell. In the present study, changes in the receptor (i.e., pattern of distribution and mobility, rate of shedding, and re-expression) following interaction with dextran or oxidized dextran will be monitered in the normal and neoplastic cell pair. An attempt will be made to integrate the receptor dynamics induced by dextran or oxidized dextran with alterations in cell function, i.e., protein synthesis and secretion, differentiation, DNA synthesis and mitosis. A causal relationship between the receptor dynamics and cell function will be tested by introducing dextran or oxidized dextran directly (without receptor perturbation) into the cell via a unilamellar lipid carrier vesicle.

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154. CHEMISTRY AND MEASUREMENT OF INTERCELLULAR ADHESION

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We have identified dual adhesion systems in the surfaces of chick embryonic retina and optic tectum cells, and have prepared antibodies and adhesion-inhibiting Fabs directed against each. The two systems are mutually non-recognizing and could in principle function as coordinate systems for Cartesian mapping of retina onto optic tectum. We are now determining whether these systems are distributed in gradients on the retinal and tectal surfaces. We are also attempting to prepare monoclonal antibodies against each adhesion system. With these, we hope to resolve the cell surface localization of each of the two adhesion systems at the ultrastructural level.

We are continuing our study of the guidance of amphibian pronephric duct migration by a wave of changing adhesiveness that, according to our evidence, passes posteriad across the embryonic flank at precisely the speed at which new somites become segmented.

We are seeking a role for fibronectin in normal morphogenetic processes.

Finally, we are applying our newly developed Cahn balance/parallel plate compression procedure to the physical measurement of sigma's (cohesive energies) of embryonic cell aggregates of several kinds.

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155. LYSOSOME BIOGENESIS - NORMAL AND TUMOR CELLS

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The extracellular levels of lysosomal enzymes have been implicated in the development of uncontrolled cellular growth, tumor invasiveness and the inflammatory response. Previous work in our laborstory has uncovered a mannosyl/glucosyl specific receptor system associated with macrophage plasma membranes which mediates adsorptive pinocytosis (uptake) of lysosomal glycosidases and glycoproteins having mannose in the terminal position. The receptor also recognizes glucose and N-acetylglucosamine but not galactose. We propose to study the mode and mechanism of uptake of lysosomal enzymes by macrophages. The regulation of the receptor will be studied by following ligand binding and by preparing receptor-specific antibody. The interrelationships between ligand and internalization and lysosomal enzyme biosynthesis and turnover will be investigated.

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156. GROWTH FACTORS AND RECEPTORS IN CHEMICAL TRANSFORMATION

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The specific alterations which occur in the control of cell proliferation in chemically transformed cells will be explored in this project. Data obtained in this laboratory using the nontransformed AKR-2B and C3H/10T1/2 mouse embryo derived cell lines and the chemically transformed derivatives of these cells, called AKR-MCA and C3H/MCA-58, respectively, have indicated that most of the characteristics of the transformed cells could be accounted for by continuous mitogenic stimulation with a growth factor. Both of the nontransformed cells have epidermal growth factor (EGF) receptors and are stimulated to proliferate by EGF. The transformed cells are not stimulated by EGF and have no detectable EGF receptors. Preliminary studies indicate that the AKR-MCA cells secrete a factor (or factors) into serum free medium which stimulates cell proliferation in the resting nontransformed AKR-2B cells and which competes for EGF binding on these cells. The studies in this project are designed to test the hypothesis that autostimulation of cells by growth factor which they themselves produce may be one mechanism in the chemical transformation of cells. This will be accomplished through the identification and attempted purification of growth factor-like substances produced by the chemically transformed cells (AKR-MCA and C3H/MCA-58) and not by the nontransformed cells (AKR-2B and C3H/10T1/2). Partially purified or 'purified' growth factor will be charcterized with respect to its biological and chemical properties. Changes in the EGF receptors of nontransformed cells at various stages of the cell cycle and with continuous mitogenic stimulation with growth factors that do not compete for this receptor will be examined. It will also be determined whether the inability of the chemically transformed cells to bind 125I-EGF is due to occupancy of the receptor by a secreted growth factor, masking of the receptor by other cell constituents, or rapid inactivation (down regulation) of receptor. The proposed experiments should yield data valuable in proving or disproving the hypothesis.

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157. PDGF RECEPTORS - IDENTIFICATION AND PROPERTIES

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Platetet-derived growth factor (PDGF) promotes DNA synthesis by 3T3 cells, diploid fibroblasts, and smooth muscle cells. The objectives of this project are to radiolabel purified PDGF with 125I, to identify specific cellular PDGF receptors by study of 125I-PDGF binding, and to examine PDGF receptor expression by normal and virally transformed cells. Homogeneous PDGF will be prepared from clinically outdated platelets. Iodine-125 will be introduced into PDGF via the chloramine-T lactoperoxidase, or 125I-containing N hydroxysuccinimide reagent procedure. Iodinated PDGF will be tested for bioactivity prior to use in the receptor assay. To identify specific receptors, binding of 125I-PDGF to Balb/c-3T3 cells will be

measured. Saturation analysis, Scatchard analysis, and competition studies will be performed. Binding specificity for different cell types will be studied. Cells will be tested for possible modulation of receptors in the absence of PDGF. PDGF binding to virally transformed cells will be compared with binding to normal cells. This project will relate binding of PDGF to the initiation of proliferation of Balb/c-3T3 cells.

158. STUDIES ON THE CELL SURFACE RECEPTOR FOR IGE

Metzger, H., Kanellopoulos, J., Fewtrell, C., Holowka, D., Goetze, A., Rivnay, B., Kempner, E., Liu, T. Y., Gitler, C., Berovici, T., Sobotka, A., Lichtenstein, L., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Inst. of Arthritis Metabolism & Digestive Diseases, Chemical Immunology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

We are pursuing an in-depth investigation into the structure and function of the membrane receptor for IgE. For these studies, intact cells from a rat basophilic leukemia, as well as normal rat mast cells, are being used. In addition, receptor-bearing membrane vesicles and solubilized receptors are being studied. The aim is to understand the structure of the receptor and its integration in the plasma membrane. Results of such studies, as well as direct functional studies, are aimed at understanding the early stimulation of cells by antigen-induced, antibody-mediated reactions.

BIBLIOGRAPHIC REFERENCES: Holowka, D., Hartmann, H., Kanellopoulos, J. and Metzger, H.: Association of the receptor for immunoglobulin E with an endogenous polypeptide on rat basophilic leukemia cells. J. Receptor Res. (In press) 1980. Dessaint, J-P, Waksman, B.H., Metzger, H. and Capron, A.: Cytophilic Binding of IgE to the macrophage. III. Involvement of cyclic GMP and calcium in macrophage activation by dimeric or aggregated rat myeloma IgE. Cellular Immunol. 51: 280-292, 1980.

159. A MORPHOLOGIC INVESTIGATION OF VIRUS INDUCED MEMBRANE RECEPTORS

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Herpes simplex virus infected cells develop a receptor capable of binding the Fc portion of IgG at the same time viral membrane antigens appear; it is not known whether this Fc receptor is a host cell or viral protein, though the latter is more likely. Studies utilizing hemocyanin-tagged IgG to label the Fc receptor and ferritin-tagged anti-HSV antigens on virally infected cells will determine whether the Fc receptor co-labels with antibodies to HSV antigens.

160. ISOLATION AND CHARACTERIZATION OF FC RECEPTORS

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The objectives of this proposed research are to isolate Fc receptors from cultured cell lines, and to characterize these receptors by immunologic and biochemical methods. Fc receptors are found on a wide variety of different cell types, and while it has been speculated that they function in the initiation and regulation of the immune response, this role has not been specifically defined.

The murine leukemia, L1210, provides a homogeneous and abundant source of Fc receptor bearing cells. Two approaches of Fc receptor isolation will be used. One involves the redistribution (capping) and release of Fc receptors by the use of immune complexes bound to the cell. The other approach is to isolate Fc receptors by affinity chromatography from plasma membranes prepared by a two-phase system of polyethylene glycol and dextran. The isolated products will be analyzed by various biochemical methods in order to determine their composition and structure. Immunologic characterization will involve studying antigenic similarities and differences of Fc receptors from different cell types.

161. BIOCHEMICAL STUDIES OF A MAJOR OPSONIC PROTEIN

Molnar, J., Gabelman, L., Chevalier, D., Ng, P., University of Illinois, Medical Center at Chicago, School of Basic Medical Sciences, Dept. of Biological Chemistry, *1853 W. Polk St., Chicago, Illinois*, 60680, United States of America

Our purpose is to investigate the roles of fibronectin and heparin in phagocytosis of gelatin-coated neutral colliods. We are using 125I-gelatin coated latex particles (g-Ltx) in conjunction with rat peritoneal macrophages. Our major aims for the next period are: 1) To establish the important kinetic parameters involved in the phagocytosis of g-Ltx. 2) To study the involvement of fibronectin in the phagocytosis of tissue debris and determining factors. 3) To investigate the nature of macrophage receptors in relationship to Fc and C3b receptors. 4) To study the nature and role of phosphorylation of chicken surface fibronectin from fibroblast by protein kinases.

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162. CELL CYCLE DEPENDENCE OF CELL SURFACE RECEPTORS

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Our major objective is to determine the mechanism and physiological role of cell cycle dependency of MSH receptors and to investigate how the responsiveness of melanoma cells to MSH is regulated by growth controls. Cell cycle and MSH-receptor content of melanoma cells, cultured in serum-containing and chemically defined medium will be analyzed by microfluorimetry and flow cytometry of cells, labeled with MSH. The effect of growth controls on the expression of MSH receptors and hormone-induced melanogenesis will be studied. Also, we want to develop a method to prevent cell cycle-dependent evasion of melanoma cells from site-directed (MSH-receptor mediated) chemotherapy of melanomas.

E. OTHER STUDIES ON CHARACTERIZATION OF SPECIAL CELL SURFACE ANTIGENS

163. GENETIC ANALYSIS OF THE PLASMA MEMBRANE IN MAN USING CELL HYBRIDS

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A genetic analysis of the human cell surface will be accomplished at three levels: 1) external membrane proteins, 2) plasma membrane glycoproteins, and 3) membrane antigens. The external membrane proteins and glycoproteins will be labeled by standard procedures and analyzed by two-dimensional electrophoresis. Membrane antigens will be analyzed using the lymphocyte hybridoma strategy in which monoclonal antibodies are produced against human surface determinants. The expression of each of these three types of plasma membrane components will be ascertained on: 1) human cell lines derived from different tissues, 2) human cell lines from individuals with inherited metabolic diseases in which the plasma membrane may be altered, 3) transformed human cell lines, and 4) neoplastic human cell lines. The normal and abnormal electrophoretic and/or antigenic phenotypes determined for the plasma membrane components on these different cell types will be investigated in rodent-human cell hybrids. After fusion of the different human cell types with mouse cells the somatic cell hybrid methodology will permit dissecting the components of the plasma membrane and the determination of chromosome assignments of genes coding for human plasma membrane proteins, glycoproteins, and antigens. This research will characterize the plasma membrane phenotypes of a variety of different human cell types, determine if the phenotypes are of a genetic origin, and map human

plasma membrane genes. Such information is important for understanding the genetic organization and biogenesis of the cell surface.

164. IA-LIKE ANTIGENS AND OTHER MEMBRANE GLYCOPROTEINS IN HUMAN LEUKEMIA

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OBJECTIVE: To use immunologic reagents to characterize and follow the progress of patients with chronic and acute leukemias. Reagents will include Ia-like anti-sera and sera against other developmentally related surface proteins.

APPROACH: Standard techniques of immunofluorescence and surface protein radiolabelling and analysis.

165. STRUCTURAL AND SEROLOGICAL STUDIES ON IA ANTIGENS

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The Ia alloantigens are the only isolated gene products of the immune response control region (I region) of the mouse major histocompatibility complex (MHC). The molecules bearing Ia alloantigenic determinants have been implicated in the control of immune response function by experiments showing that anti-Ia alloantisera specifically inhibit such functions. Furthermore, the Ia structural genes are arranged so that the Ia molecules could provide a molecular explanation for Ir gene complementation effects. Since Ia antigens may have an active role in the control of immune response, these molecules must be analyzed to see if their structural features can explain how they carry out their proposed function.

The research projected here includes structural, genetic and serological investigation of Ia molecules. Structural analysis will include assessment of variability in primary structure, primarily by peptide mapping, localization of antigenic sites, search for interaction with other cell proteins, and mechanism of assembly and transport of Ia molecules to the cell surface. Genetic analysis will include a survey of available recombinants to further define the arrangement of Ia structural genes. Serological analysis will mainly involve a search for antibodies to unassembled Ia chains, or to all antigenically silent Ia molecules or chains.

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166. PHYSICOCHEMICAL CHARACTERIZATION OF LYMPHOMA-ASSOCIATED ANTIGEN (LAA)

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OBJECTIVE: Physicochemical characterization of lymphoma-associated antigen (LAA) in lymphoma patients.

APPROACH: The physicochemical characterization including such parameters as molecular weight by SDS polyacrylamide gel electrophoresis, sedimentation coefficient by differential gradient centrifugation, migrating property by immunoelectrophoresis and homogeneity by isoelectric focusing will be carried out. The chemical nature of the protein and its subunits, and their molecular weight and amino acid sequence will also be determined.

167. ANALYSIS OF THY-1 POLYMORPHISM IN DIFFERENT CELL TYPES

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Low molecular weight membrane glycoproteins which carry Thy-1 alloantigenic sites can be isolated from certain rat and mouse cells at particular stages in their differentiation. Structurally homologous molecules, which lack the Thy-1 alloantigen, have now been identified in the tissues of dog and man, and these also exhibit a highly restricted tissue distribution. In each of the four species so far examined, Thy-1 glycoproteins isolated from brain tissue resolve into two bands of SDS-PAGE; similarly Thy-1 extracted from in vitro cultures of human, murine and rat embryonic fibroblasts resolve into two bands which have higher apparent molecular weights than the corresponding brain Thy-1 molecules. Thus Thy-1 glycoproteins exist in each of the four animal species in polymorphic forms which can be readily distinguished from each other and which reflect their tissue of origin. The molecular basis of this polymorphism is being investigated by examination of fibroblasts derived from single cell clones, by studying the glycosylation process and by examination of distribution of Thy-1 on different cell types within the brain. Preliminary results suggest that the human brain astrocyte may express a single molecular species.

168. THY-1 ANTIGEN - LATENT ANTIGEN ALLOWS RAPID EXPRESSION ON THE CELL SURFACE DURING DIFFERENTIATION

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Thy-1 differentiation antigen is a glycoprotein (molecular weight approximately 18,000) found on cell surfaces in various tissues (lymphoid, hemopoietic, nervous and mammary) at certain stages of their differentiation. Antigenic determinants are on the protein component of the molecule and are of two main types: xeno and allo, recognized by xeno- and allo-antisera, respectively. Both are present on a single molecule.

Enriched populations of thymocyte precursors (prothymocytes) have been obtained by BSA density gradient centrifugation. These cells have some partially exposed Thy-1-molecules (xeno determinants only) at the cell surface (anti-xeno antibodies bind to the cells, anti-alloantibodies do not). Additional Thy-1 is totally hidden (unavailable to antibody) but is uncovered by detergent solubilization. Moreover, treatment of prothymocytes with dibutyryl cyclic AMP, which mimics in vivo induction with thymic hormones, leads to the rapid (20 min.-3 hours) exposure of allo-antigenic determinants typical of mouse thymocytes. The function of such 'latent' antigen may be to provide rapid changes in the cell surface during differentiation, thus avoiding delay involved in de novo synthesis and membrane insertion of surface molecules.

'Latent' Thy-1 may also be involved in a second system. Unactivated rat platelets are Thy-1-negative. However, very early in activation Thy-1 becomes rapidly exposed on the platelet surface. This exposure may be due to platelet shape change whereby Thy-1 that was concealed within canaliculi (surface invaginations) is uncovered as these evert.

169. PURIFICATION AND STRUCTURAL CHARACTERIZATION OF GLYCOPROTEINS RELATED TO THY-1

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Thy 1:1 and Thy 1:2 are differentiation alloantigens expressed on certain rat and mouse cells. We have purified low molecular weight, integral, plasma membrane glycoproteins which carry these alloantigens by a procedure which involves chloroform-methanol delipidation of homogenized mouse brain, detergent extraction in sodium deoxycholate and affinity chromatography on lentil-lectin sepharose. Pure Thy-1 glycoproteins were isolated from the lectin-bound fractions by successive gel filtration on Sephacryl S-200 columns equilibrated in sodium deoxycholate or urea/NP40. The purified molecules from both AKR(Thy 1:1) and CBA(Thy 1:2) brain run as doublets on polyacrylamide gel electrophoresis in sodium dodecyl sulphate (SDS PAGE), with apparent molecular weights of 25,100 and 25,300 (AKR) and 25,800 and 25,400 (CBA). Amino acid composition data suggest that there are only minor amino acid differences between the two allotypes.

Rabbit antisera raised against purified Thy-1 glycoproteins from rat or mouse recognize additional species-specific and species-cross-reactive determinants on the molecule. We have used the species cross-reactive component of such antisera to identify homologous glycoproteins in human brain. The human Thy-1

homologue also runs as a doublet on SDS-PAGE, with apparent molecular weights of 26,400 and 25,800, and has a remarkably similar amino acid composition to that of rat and mouse Thy-1. Following reduction, alkylation and succinylation the molecule can be efficiently cleaved with trypsin and Staphylococcus aureus V8 protease and the peptides isolated by high pressure liquid chromatography. The amino acid compositions of these peptides have been compared with those of similar peptides derived from rat brain Thy-1. Such comparisons indicate that the Thy-1 glycoprotein is strongly conserved between species.

170. CHARACTERIZATION OF THE PHOSPHORYLATION SITE OF THE HLA-DR ANTIGENS

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The overall aim of this research is to characterize the phosphorylation site of the HLA-DR antigens, the human histocompatibility antigens located at the surface of cells of the immune system. This investigation will examine the role of phosphorylation in the regulation of movement of the DR antigens in the plasma membrane. The mechanism of regulation involves binding of the antigen to the cytoskeletal elements composed of actin. This movement has previously been shown to be associated with immune functions involving recognition and induction of proliferation.

The proposed research involves chemical cleavage of the DR polypeptide and isolation of the phosphate containing fragments which will then be sequenced. The actin binding properties of the HLA DR will be studied using competetive binding studies with myosin, spectroscopic chemical labels, chemical crosslinking and ultracentrifugation. The structural features of the putative actin binding site will then be characterized and the mechanism of its regulation by phosphorylation will be determined.

171. PRIMARY STRUCTURE OF MHC I REGION ASSOCIATED ANTIGENS

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Proteins encoded by the I region of the major histocompatibility region (Ia antigens) will be isolated from Ia-bearing tumor cells. The cells will be radiolabeled with 3H, 35S and/or 14C amino acids, detergent solubilized and isolated with immunoadsorbants, utilizing monoclonal anti-Ia alloantisera from hybridomas. Protocols for isolating cyanogen bromide and proteolytic fragments from both the alpha and beta chains of the Ia molecules labeled with a variety of 3H amino acids and 35S cys will be initially developed. These fragment isolation procedures will be designed to maximize the recoveries of the radiolabeled products. Once the peptide isolation procedures are established, Ia molecules will be uniformly radiolabeled with 14C, the peptides isolated and sequenced.

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172. STRUCTURES OF HISTOCOMPATIBILITY-2 MEMBRANE ANTIGENS

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The objective of this proposal is to characterize the structures of H-2 antigens. This study will focus primarily on the conformations of the membrane-bound glycoproteins coded for by H-2k and H-2K regions of the major histocompatibility complex of the mouse.

The research plan will be directed in essentially four areas: 1) Several methods will be tested for improving the yields and separation of H-2b antigens as presently isolated in highly purified form from EL-4 cells by a purification scheme developed, in part, by the Principal Investigator. The aim here is to purify H-2b antigens from this lymphocytic leukemia-derived cell line for structural examination by conformationally sensitive spectroscopic probes, such as circular dichroism and fluorescence, and by amino acid composition and sequence analysis. 2) In an extension of current studies by the Principal Investigator, various molecules with public antigenic specificities of the H-2.28 family will be compared by tryptic peptide map analysis. The aim is to clarify the structural basis of this complex family of antigens which has recently led to the discovery of a new class of H-2D antigens. 3) The nature of the antigenic sites of H-2 antigens will be explored by chemical reagents which alter serological reactivity. Peptides protected from modification by specific alloantibody, and therefore presumably in or near antigenic sites, will be identified by tryptic peptide mapping techniques.

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173. STRUCTURAL STUDIES OF THE PRODUCTS OF THE H-2 COMPLEX

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The overall objective of the research proposed in this application is the elucidation of the detailed structure of two sets of gene products encoded by the H-2 complex, the major histocompatibility complex (MHC) of the mouse. The gene products to be studied, the H-2K and Ia antigens, are both highly polymorphic sets of glycoprotein molecules integrated in the lymphocyte plasma membrane. The studies proposed in this application should lead to a thorough knowledge of the primary structure of both the protein and carbohydrate portions of these antigens. For the H-2K antigens, the proposed studies involve large scale biochemical purification, amino acid sequence determination, and carbohydrate structure determination. For the Ia antigens, biosynthetic radiolabeling will be combined with immunochemical purification; the materials thus isolated will have their amino acid sequences and carbohydrate structures determined by radiochemical methods. Comparative analysis of amino acid sequence data (employing data obtained in other laboratories as well as our own) will be exploited to try to deduce the antigenically significant portions of these molecules. In addition, experiments will be carried out which approach this problem directly, either through the use of tryptic or cyanogen bromide fragments (of known structure) to inhibit the binding of alloantisera to intact antigens, or through the use of alloantisera to block reaction of protein modification reagents with the antigenic site.

Cross-linking studies will be carried out to try to elucidate interactions of H-2 and Ia antigens with other molecules in the lymphocyte plasma membrane. Finally, experiments will be undertaken to try to establish the molecular function of the H-2 antigens in 'H-2 restricted' anti-viral cytotoxicity; and to examine the function(s) of the Ia antigens in I region regulated antigen presentation systems in vitro.

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174. STRUCTURAL ANALYSIS OF MURINE H-2L AND H-2O ANTIGENS

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The proposed studies involve the structural characterization of two relatively recently defined murine transplantation antigens, H-2L and H-2O. These molecules have been detected in the D region of mouse strains of the d and q haplotypes. The H-2L and H-2O transplantation antigens will be isolated from

detergent lysates of intrinsically radiolabeled cells by sequential immunoprecipitation with appropriate antisera or monoclonal antibodies. Initially tryptic peptide map comparisons will be made to determine the relationship of the H-2O and H-2L gene-products to each other and to other previously described transplantation antigens especially the H-2D molecule of the same haplotype. Further structural characterization of the H-2O and H-2L molecules will include amino acid sequence determinations as well as determination of the number of glycosyl groups and number and location of disulfide loops. Structural studies on these H-2D region gene products should shed light on the evolutionary relationships of H-2 antigens, discern whether there are structural features that distinguish these antigens from H-2K and H-2D antigens and provide additional information on the structural basis for the extensive polymorphism of transplantation antigens which has been observed in serological studies.

175. HLA-DR-LIKE ANTIGENS ON HUMAN MILK FAT GLOBULE MEMBRANE AND BREAST EPITHELIUM

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The milk fat globule membrane (MFGM) originates from the mammary epithelium during lactation by enveloping globules of fat during the secretory process. Suspensions of MFGs were positive when tested with a rabbit anti-DR serum or mouse hybridoma monoclonal anti-DR antibodies (DA2).

Examination of formalin-fixed preparations of breast tissue showed DR antigens to be localized on the secretory epithelium, although these were found only on lactating breast sections. Non-lactating breast, carcinomas or benign tumors, cultures of normal breast epithelium (R.C. Hallowes), a pleural effusion line MCF-7 or epithelial cells from milk (S. Chang) were negative.

Antisera raised against MFGM after absorption with epithelial membrane antigen (EMA) possessed residual anti-DR activity as judged by their ability to bind to DR-positive lymphoid cells or to precipitate two bands of c. 34,000 and 23,000 daltons from radio-labelled lymphoid DR-positive cell lines.

Further investigations will establish whether DR antigens from hemopoietic origin differ from those of epithelial origin and it is hoped that a large scale purification of DR antigens from MFGM will also be possible.

176. CHARACTERIZATION OF THE FELINE ONCORNAVIRUS-ASSOCIATED CELL MEMBRANE ANTIGEN INDUCED BY FELINE SARCOMA VIRUS TRANSFORMATION

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The aim of this project is to further characterize the feline oncornavirus-associated cell membrane antigen (FOCMA) expressed on feline sarcoma virus (FeSV) transformed cells. Specifically, (1) the expression of FOCMA on nonproducer FeSV transformed cells of various species was defined by membrane immunofluorescence, (2) FOCMA was characterized biochemically to determine its size and whether there were determinants unique from the virus structural proteins, (3) hybridomas producing monoclonal antibody to FOCMA were developed in order to purify the protein and to employ the antibodies in an immunotherapy program for feline leukemia, and (4) to determine if FOCMA is a true tumor specific antigen and whether it is encoded by the virus and induced upon transformation independently of virus replication.

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177. LYMPHOCYTE ANTIGENS - STRUCTURE, FUNCTION AND SYNTHESIS

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The objective of the research project is to identify and characterize surface glycoproteins of mouse lymphocytes with the long-term goal of understanding lymphocyte differentiation and function. Monoclonal antibodies have been obtained against several mouse lymphoid tissue-specific surface glycoproteins including Thy-1 and T200 glycoproteins. These reagents are being used to study various aspects of hematopoietic differentiation in the mouse and to investigate the biosynthesis of specific cell surface molecules. Efforts to obtain monoclonal antibodies which can be used to biochemically detect surface glycoproteins restricted to specific subpopulations of mouse lymphocytes will continue. Such antibodies will be characterized on a panel of mouse hematopoietic cell lines and selected antibodies then used in genetic, biochemical and functional studies of mouse lymphocyte cell surfaces.

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178. MEMBRANE COMPONENTS OF NORMAL AND LEUKEMIC LEUKOCYTES

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The project will focus on the structure and function of leukocyte membrane receptors and surface antigens. The project has three interrelated long-term objectives. The first of these is to characterize normal leukocyte membrane surface markers for their distribution on different leukocyte types and for their order of appearance during leukocyte maturation. The project will not be restricted to one leukocyte type, but will encompass the spectrum of bone marrow nucleated cells, including erythroid precursors and megakaryocytes. The findings on normal leukocytes form the basis for the second objective of the project, to find and characterize leukemia-specfic membrane markers and determine which of these may have prognostic significance for early determination of important clinical subtypes or stages of leukemic disease. The third goal is to analyze lymphocyte membranes by scanning electron microscopy and determine the distribution and association of different types of receptors on individual cells.

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179. CHARACTERIZATION OF HUMAN B LYMPHOCYTE DIFFERENTIATION ANTIGENS

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The research project is aimed at more precise definition of surface membrane markers for B lymphocyte differentiation in the human. A second goal is the identification of membrane components involved in activation of B-cells for antibody synthesis. Finally, a model for the development of B lymphocyte neoplasia will be tested with the markers developed in the initial phase of the study.

The methods involved in the proposal are the preparation and testing of conventional and monoclonal antibodies based on strategies developed in our parallel work on the mouse and the concept that there will be a high degree of molecular homology between murine and human differentiation antigens. Binding

studies and correlation of new markers with known B-cell surface receptors will be done using two-color analysis on the dual laser fluorescence activated cell sorter. Membrane components will be characterized using two-dimensional gel electrophoresis. Normal human B-cells, as well as lymphocytes from chronic lymphocytic leukemia and lymphoma patients, will be analyzed for the sequence of appearance of B-cell markers and their presence or absence during pre-neoplastic states. Assuming we identify B-cell receptors for growth-regulatory factors, a hypothesis which states that an initial mutation leads to a loss of regulation of cell proliferation because of the absence of a membrane receptor class can be tested. The role of antigen in the unregulated growth process will also be tested with anti-idiotypic antibodies to characterize clonal expansion.

180. IMMUNOCHEMICAL CHARACTERIZATION OF NEURONAL CELL SURFACE ANTIGENS

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The nerve cell surface presumably plays a critical role in nerve function and in nerve cell-cell recognition and may therefore reflect the pathways of differentiation of distinct nerve cell types. Schachner and co-workers have prepared a series of distinct antisera that specifically detect antigens unique to the cell surface of murine nervous tissue. I propose to biochemically characterize and isolate antigens detected by two of these antisera, designated NS-1 and NS-3. Tissue cultured cell lines bearing NS-1 or NS-3 antigens will be radiolabeled by cell surface labeling techniques specific for protein or carbohydrate and NS-1 or NS-3 antigens will be isolated from detergent solubilized cell fractions using NS-1 or NS-3 antisera. Antigens isolated by electrophoresis or thin layer chromatography will be used to generate more specific antisera that will facilitate biochemical identification and immunological fractionation of differentiated nerve cell types. These more specific antisera will also be useful in analyzing nerve cell-cell interactions in living nerve cells.

181. IMMUNOGLOBULIN A AND THE HUMAN GASTROINTESTINAL TRACT

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Our immunocytochemical studies on normal human colonic epithelium have demonstrated that two glycoproteins on the cell surfaces are distributed in a polar manner: Secretory component (SC), the membrane receptor for polymeric immunoglobulins, is located exclusively on the apical membrane. In colonic adenocarcinoma, SC is reduced in amount or absent, and CEA is expressed over the entire cell surface.

We propose to examine further the biological and therapeutic implications of disturbances in the polar distribution of membrane surface antigens. The specific aims are: (1) Determine the specificity and extent of alterations in cell surface markers in neoplastic and inflammatory diseases of the large bowel and other organs. Various epithelial tumors and tissues from inflammatory bowel diseases will be examined for distributional changes in CEA and SC as well as in enzyme markers (alkaline phosphatase and K ion-dependent phosphatase) of the cell surface domains. (2) Produce an animal model for study of the abnormal distribution of surface membrane components in neoplastic transformation of colon epithelial cells. Two markers of the basolateral membranes of rat colon epithelial cells (SC and K ion-dependent phosphatase) and two markers of the apical membrane (alkaline phosphatase and an immunocytochemically indentifiable marker) will be localized. Alterations in surface distribution of the membrane markers during neoplastic transformation induced by dimethylhydrazine will be monitored. (3) Utilize redistributed surface membrane components in the therapy of experimentally induced colonic cancers. Colonic tumors will be induced in rats by dimethylhydrazine, and an attempt will be made to destroy the tumor cells by injected heteroantibodies directed specifically against apical membrane components aberrantly expressed on basolateral surfaces.

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182. CELL SURFACE GLYCOCONJUGATES IN PANCREATIC CANCER

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As part of the continuing investigation of cell surface glycoconjugates in pancreatic cancer, the following studies will be carried out: 1. The development of monoclonal antibodies to human pancreatic cancer cell lines using the mouse hybridoma system will be continued. The antibodies will be screened and suitable ones will be used to develop a radioimmunoassay to detect pancreatic carcinoma using samples of human serum. Suitable antibodies will also be used to purify and characterize the cell-surface antigens of the human cell lines. 2. The cell-surface antigens of human pancreatic cancer cells in tissue culture will be compared with those of solid tumours produced in nude mice by the same cells. 3. The investigation of the effects of differentiating agents on the cell-surface components and also on the tumourigenecity of the human pancreatic cancer cell lines will be expanded. 4. The interaction of the cells with extracellular matrices will also be examined in an attempt to understand the processes of invasion and metastasis.

183. MEMBRANE ANTIGENS OF RAT PROSTATE ADENOCARCINOMA

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Two different sublines of the Dunning R3327 transplantable prostate adenocarcinoma are being investigated. These sublines, designated G and H at our institution, demonstrate in vivo and in vitro differences. The G subline is rapidly growing and poorly differentiated histologically, while the H subline grows very slowly and is a well-differentiated tumor.

Lectin binding studies have demonstrated quantitative differences between the two tumors. Purified membrane preparations from the H tumor bind more concanavalin A (Con A) and wheat germ agglutinins than do G tumor membrane preparations. These studies have been extended to monolayer cell cultures derived from G and H tumors. The H tumor cells bound more Con A than did the G cells, and further analysis of these kinds of experiments suggests a heterogeneity of binding sites of both G and H cells in culture.

Tumors and cells in culture analyzed by flow cytometry have demonstrated that the tumors consist of at least two populations of cells, one diploid and one aneuploid. Cell separation techniques and cloning experiments are underway to identify the tumor cell.

Antibody has been produced in goats to purified membranes of the G and H tumors and in rabbits to whole tumor cells. Fractionation of the immunoglobulins and affinity chromatography have resulted in the isolation of an antigen. The tumor specificity of this antigen is under investigation.

184. ISOLATION AND CHARACTERIZATION OF HETEROPHILE ANTIGENS

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This study will be primarily concerned with the isolation and characterization of the following heterophile antigens: infectious mononucleosis heterophile antigen (Paul-Bunnell antigen, PBA), Hanganutziu-Deicher antigens, and heterophile transplantation antigens. These antigens are detected on bovine erythrocyte membranes and can be extracted in soluble form. PBA activity is found in the minor glycoprotein fraction of bovine erythocyte membranes and has lipid-like properties. PBA will be further purified and its composition determined. Studies on amino acid sequence and oligosaccharide sequence and linkage will be carried out. PBA-like substances will also be studied on normal and malignant human lymphoid cells.

Hanganutziu-Deicher antigens (HDA) appear in the sera of patients with various malignancies, lymphoma, leukemia and other pathologic conditions. The high molecular weight glycoprotein fraction of bovine erythrocytes contains HDA that are related to the antigen found in human sera. This fraction, which contains several other components, will be further fractionated. The structure of purified HDA will be examined and the antigenic determinants defined. The neutral glycolipid fractions of bovine erythrocyte membranes contain substances that are reactive with heterophile transplantation antibodies. The identity of these substances will be established and their structures determined.

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185. HUMAN BLOOD AND TISSUE PROTEINS

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Human macrophages and what appears to be a small population of thymic lymphocytes, react with unsaturated fatty acids (20:4 and C22:6). These lymphocytes will be defined in terms of known surface antigens. Rat alpha-fetoprotein derived from fetuses, maternal and hepatoma sera will be studied as to their variable contents of unsaturated fatty acids and their effects on the inhibition of phytohemagglutinin stimulated transformations of rat peripheral lymphocytes. The binding constants of both the human and rat source proteins with various fatty acids will be further explored. The sequence studies of human alpha-fetoprotein will be extended.

The sequence of the WEIR lambda-chain will be determined since its dimer with the MCG L-chain can be crystallized in a form suitable for X-ray crystallographic studies. The sequences of these two lambda-chains will be related to the structural interactions in the crystalline hybrid protein and compared with results obtained for the MCG dimer. The influence of dimer formation between the WEIR and MCG L-chains on the immunological properties exhibited by the Bence-Jones forms of each protein will be studied and related to the chain interactions determined by X-ray diffraction. Work on defining the relationships of sequence to serologic factors (Mcg, Kern, Oz, St, 111) in various lambda chains will be extended.

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186. URINE AS A SOURCE OF HUMAN CELL SURFACE MARKERS

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The major objective will be to elucidate the molecular structure of the allotypic sites of human histocompatibility antigens. To this end, highly purified antigens expressing different phenotypes will be selectively cleaved with proteases and antigenically reactive peptides will be isolated by affinity chromatography with monoclonal antibodies. Such peptides will be analyzed for molecular size and amino acid sequence to determine their location within the overall molecular structure of histocompatibility antigens and to delineate the specific molecular structures which are associated with known allotypic specificities of human histocompatibility antigens.

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187. CELL SURFACE CHANGES IN TRANSITIONAL CELL TUMORS

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The proposed studies explore the possibility of a relationship between changes in the cell surface properties of transitional cell carcinomas (TCC) of the urinary bladder and the clinical course of the disease. Detectability of ABH and T antigens, agglutinability by lectins, and sialic acid content of the tumors will be correlated with biological behavior (in particular, frequency of recurrences, invasiveness, and metastases) in an attempt to assess the prognostic significance of such parameters. The mechanism by which cell surface changes are brought about will be studied by following the activities of enzymes involved in the synthesis of ABH substances (glycosyltransferases) and their degradation (glycosidases). Tumor and serum sialyltransferase activities and sialic acid levels will be tested to investigate possible correlations with the course of the disease. It is hoped that these studies will provide important information about the biological behavior of TCC and may provide a means of assessing the prognostic outlook of patients with such tumors.

188. CELL SURFACE DIFFERENTIATION DURING GERM CELL DETERMINATION

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We plan to study the process of germ cell determination by examining the differentiation of that portion of the egg cell surface that is incorporated into the germ cells. We will be using Drosophila melanogaster as the experimental system for several reasons: 1) Methods are available for large-scale preparation of pure primordial germ cells; 2) The descriptive embryology of D. melanogaster is very extensive; and 3) Maternal-effect mutants in germ cell determination are available for study. The study will involve the following general steps: 1) A descriptive study of the surface proteins and glycoproteins of primordial germ cells and early somatic cells; 2) Preparation of antiserum directed against germ cell-specific suface antigens; 3) Preparation of monospecific antisera from this antiserum; 4) Ultrastructural localization of germ cell-specific surface antigens on oocytes, eggs, and embryos of wild type and mutant flies, and 5) Quantitative analysis of germ cell surface antigen distribution.

189. GENETICS AND BIOCHEMISTRY OF MOUSE ALLOANTIGENS

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I plan to investigate a segment of mouse chromosome 17 situated near the major histocompatibility complex (MHC). The objectives are to elucidate the organization of genetic information; define the extent of polymorphism and/or number of loci encoding cell surface antigens in this region; determine the expression of these antigens on various populations of lymphocytes in order to identify markers of functional subsets of lymphocytes; then investigate the biochemical nature of these antigens to determine their evolutionary relationship. In order to accomplish this, I will initiate experiments to search for genetic recombination in this region. These and other recombinants will be serologically analyzed by cross-immunization and absorption of antisera. The biochemical characteristics will be established by immunoprecipitation of antigen and analysis by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE), peptide mapping and partial N-terminal amino acid sequence analysis.

190. EMBRYONIC AND VIRALLY INDUCED TUMOR-CELL MEMBRANE ANTIGENS

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Expression of chicken fetal antigens (CFA), thirteen serologically distinct oncodevelopmental membrane antigens, will be analyzed in embryonic development and in cellular differentiation of erythroid and myeloid cells in normal and cancerous chickens, as well as in in vitro cell lines. The possibility that CFA is an important membrane receptor for cell-cell communication in embryonic development will be investigated by analyzing CFA expression on the primitive erythroid series in normal 12 hour-11 day embryos and screening for CFA abnormalities in previously identified developmental mutants. The possibility that CFA determinant expression may be associated with specific cellular differentiation-cell cycle phases will be analyzed in normal and erythroleukemic chickens, and virally transformed lymphoid cell lines. Breeding experiments will be performed in order to determine the inheritance pattern of CFA determinants. The role of genetic versus environmental factors in CFA expression will be investigated in in vitro analyses of erythroid cellular maturation of cells obtained from normal embryos and adult bone marrow. The possibility that CFA may serve as a viral receptor will be further analyzed. The relationship of AFP and suppressor T lymphocytes, both expressed during embryonic development, to suppression of the immune response system and tumor induction will be investigated.

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191. GENETIC BASIS OF ANTIGENIC VARIATION

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Tumor-associated antigens serve as potential targets for the immune system of the host and may play a role in establishing the malignant phenotype of the tumor cell. The long-term objective of the proposed research is to understand the mechanisms by which the tumor cell regulates expression of its tumor-associated antigens. To accomplish this aim we will study several cell surface antigens in detail and attempt to define and understand the mechanism of action of the genes involved in their biosynthesis and in the regulation of their expression on the cell surface. Mutants which fail to express specific antigens on their cell surface will be isolated from murine lymphomas and from cell lines expressing known biological functions. Somatic genetic analysis will be used to establish the number, dominance, and nature (structural or regulatory) of genes involved in antigen biosynthesis and control. Hybrids between tumor cells representing different states of normal differentiation will also be studied to gain further insight into the mechanisms regulating the expression of cell surface antigens.

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192. BIOSYNTHESIS OF CARCINOEMBRYONIC ANTIGEN

Mendicino, J. F., Bricker, J., University of Georgia, Franklin College of Arts & Sciences, Dept. of Biochemistry, *Athens, Georgia*, 30601, United States of America

The principal objective of the proposed research is to achieve an understanding of the pathway of biosynthesis, mechanism of secretion and function of carcinoembryonic antigen in tumor tissues. The multienzyme systems in tumors which regulate the composition, rate of synthesis and secretion of tumor antigens will be examined in structural studies and in studies of isolated cells, and cell-free particulate multienzyme systems.

Methods have been developed in this laboratory for the isolation of CEA from liver metastases of colon, pancreas, and breast adenocarcinomas in high yield. Our present efforts are being directed towards completely characterizing these purified glycoproteins. It is not yet certain what changes occur during neoplastic transformation which lead to the synthesis of CEA. In order to compare the structure of CEA with glycoproteins present in the normal cell it is necessary to have highly purified preparations of CEA. The isolation and characterization of homogenous preparations of the antigen are also required before studies on its biosynthesis in tumor tissue can be initiated. The structure of the carbohydrate moiety of the antigen must be determined before specific glycosyl acceptors for glycosyltransferases present in tumor tissue can be prepared. We then plan to examine the enyzyme systems which are involved in the synthesis and secretion of these glycoproteins in adenocarcinomas.

193. ANTIGENS OF CHEMICALLY TRANSFORMED MOUSE FIBROBLASTS

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We propose to immunize mice or rats with transformed cells, fuse their spleen cells with mouse myeloma cells and select hybridomas producing antibodies binding to the cell line used for immunization but not to normal cells. The following pairs of immunizing cells and control normal cells will be used: (1) human melanoma/autologous fibroblasts, (2) in vitro transformed human fibroblasts/parental fibroblast line, (3) in vitro transformed C3H/10T one-half fibroblasts (CL15, CL16)/ C3H/10T one-half fibroblasts (CL8), (4) UV-induced C3H fibrosarcomas/C3H/10T one-half fibroblasts. Putative transformation-associated antigens identified in this way will be studied further by binding assays with radioiodinated monoclonal antibody and by immunoprecipitation. Antigens of particular interest will be characterized structurally (peptide mapping, aminoacid sequence analysis), and their relationship to the transformed state will be determined.

194. CHEMISTRY OF TUMORICIDAL MACROPHAGE SURFACE ANTIGENS

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Macrophage cell lines have been examined for Mac-1 and Mac-2 expression and J774 and P388d1 found to express sufficient quantities for biochemical isolation. Monoclonal antibody affinity columns have been prepared with Mac-1 and Mac-2 monoclonal antibody (MAb). A HL variant of the anti-Mac-2 MAb was prepared which allowed accurate site number quantitation and tissue absorption experiments. Mac-1 has also been quantitated on both murine and human cells. Cleveland peptide mapping of the Mac-1 alpha and beta subunits shows no common peptides, suggesting an independent origin. The molecular weights of the alpha and beta subunits have been more accurately defined as 170,000 and 95,000 Mr, respectively.

195. THE MOLECULAR NATURE OF TUMOR ANTIGENS

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Our group has detected three potentially interesting cell surface antigens which appear to be tumor associated. The antigens were initially detected on human melanoma cells using specific monoclonal antibodies. Extensive testing has shown that one of the antigens (3.1) has an extremely restricted distribution. The chemical nature of this antigen has not as yet been determined. The other two melanoma-associated antigens are proteins with molecular weights of approximately 97,000 (p97) and 210,000 (p210), respectively. These two protein antigens have similar types of distribution in that each is present in many of the melanoma cell lines and in some of the non-melanoma tumor cell lines tested, but not in any of the normal cell lines tested. Many normal adult human tissues have been examined for the presence of p97 and p210 and to date neither protein has been detected in these preparations. Both p97 and p210 are now being purified by immunoadsorption procedures using monoclonal antibodies. We propose to determine the chemical and structural natures of the purified antigens. Radioimmunoassays will be developed in order to quickly screen for these antigens in normal and malignant tissues as well as in sera from cancer patients.

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Identification of a cell surface protein, p97, in human melanomas and certain other neoplasms. (1980) Proc. Natl. Acad. Sc., USA. 77:2183-2186. Brown, J.P., Wright, P.W., Hart, C.E., Woodbury, R.G., Hellstrom, K.E., and Hellstrom, I. Protein antigens of normal and malignant human cells identified by immunoprecipitation with monoclonal antibodies (1980) J. Biol. Chem. In press.

II. BIOCHEMISTRY OF CYTOPLASMIC AND EXTRACELLULAR PROTEINS

A. BIOCHEMISTRY OF CYTOPLASMIC STRUCTURAL PROTEINS

196. SYNTHESIS OF ACTIN IN AXOLOTL EMBRYOS

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We have studied the appearance of the three forms of actin in whole axolotl embryos of different stages and in early organ rudiments obtained by microdissection. The beta- and gamma-actins are found to be synthesized at all stages. The alpha-actin, normally regarded as a specific muscle protein, can first be detected by the late heat process stage (stage 25) and becomes more prominant during the subsequent tailbud stages. By stage 30 there is active synthesis in the somites, most of whose cells become the striped muscles of the myotomes, but no detectable synthesis in other regions of the embryo.

Examination of the somites by transmission electron microscopy shows that the first bundles of actin-like filaments appear also at the late head process stage. This stage lies between the time at which we consider the somites to have become fully committed to form muscle (see Report 276) and the time of the appearance of striations in light microscope preparations that coincides with the onset of motility (stage 34).

197. ACTIN ASSEMBLY IN SEA URCHIN EGGS BEFORE AND AFTER FERTILIZATION

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Cell movements and changes in cell shape often are accompanied by changes in the supramolecular form of actin. An example is the elongation of microvilli which occurs on the surface of sea urchin eggs following fertilization. Burgess and Schroeder (J. Cell Biol. 74, 1032, 1977) have recently shown that these microvilli contain actin filaments in the form of bundle, and Spudich and Amos (J. Mol. Biol., 129, 319-331) have studied the detailed substructure of these actin filament bundles by diffraction analysis of electron microscope images. Begg and Rebhun (J. Cell Biol. 79, 274a, 1978) recently reported that cortices isolated from fertilized eggs do not contain microfilaments, but that a dense network of microfilaments appears upon raising the pH to 7.5. This transformation can be readily studied since eggs can be obtained in large quantities and since about 20% of the actin of the egg is associated with the cortical layer (Spudich and Spudich, J. Cell Biol., 82, 212-226).

We will use various methods to probe the state of the actin in the cortical layer of the unfertilized egg to determine whether it is G-like, F-like, or intermediate in form. Actin from the cytoplasm, actin associated with the cell cytoplasmic surface, and that from the microvillar bundles will be characterized by various methods, including amino acid analysis to detect possible minor differences which may have resulted from enzymatic modifications. Accessory components, which may be proteins that modify the actin by binding to it or enzymatically modifying it, will be purified and characterized.

The possibility of determining the direction of growth of the filaments during microvilli elongation will be examined using the recent observation that muscle actin assembles primarily at one end and disassembles at the other (Wegner, Biophysical Chem. 7, 51, 1977).

198. ACTIN AND TUBULIN CANCER CELLS (HUMAN, ADENOVIRUS)

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To study the contractile gel associated with plasma membrane of HeLa cell., we shall purify and characterize the protein components of the gel (most likely actin and a high molecular weight protein). We shall study the responses of the purified gel components to salts, temperature, sulfhydryl blockers, and cytochalasin B and determine their influence on gel formation by purified components. We shall relate the properties of gelation in vitro to the cell by studying gelation of cell extracts during the cell cycle, investigating gelation of extracts of purified plasma membranes, identifying a newly identified component of cells and plasma membrane believed to be alpha-actinin, and studying the distribution of the gel components in the plasma membrane by light and electron microscopy. We shall also investigate gelation of extracts of normal and transformed cells.

To test the hypothesis that HeLa cells construct specific assemblies of microtubules from specific tubulin subunits, we shall first isolate microtubules by reconstitution in vitro, characterize the tubulin subunits, and determine whether MAP- of tau-like proteins are present and required for polymerization. We shall then isolate the colchicine mitotic apparatus and the intercellular bridge and determine if specific tubulin subunits are present in specific assemblies, and we shall also determine whether the assemblies contain MAP- or tau-like components.

199. ACTIN-MEMBRANE INTERACTIONS

Brown, S. S., University of Michigan, Ann Arbor Campus, Medical School, Dept. of Anatomy, *1335 E. Catherine St., Ann Arbor, Michigan,* 48104, United States of America

Although there is a large body of data suggesting that actin filaments associate with the cytoplasmic membrane, the putative binding site(s) have not been identified. A difficulty has been to design an appropriate assay. I will approach the problem with two types of assay. First, I will look for the ability of a membrane component to act as a nucleating site for actin assembly, under conditions which rule out the contribution of actin filament fragments. Second, I will look for specific binding of membrane components to actin and determine whether they bind at the end or along the length of the filament. Both of these approaches involve the use of polylysine coated beads, a new method I have developed for studying actin assembly (Brown and Spudich, 79, J. Cell Biol. 80-499).

Once a component is purified, I will examine how it is regulated and functions in the cell. I will ask whether it is in indirect contact with the external cell surface and whether it is involved in transmembrane signalling.

200. THE BIOSYNTHESIS AND METABOLISM OF DESMIN AND INTERMEDIATE FILAMENTS DURING MYOGENESIS IN TISSUE CULTURE

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The object of the experiments outlined in this proposal is to gain insights into the biosynthesis and assembly of intermediate filaments during muscle differentiation in tissue culture. In the past we have succeeded in demonstrating that the major subunit of muscle intermediate filaments is a component of myofibril Z discs. With the aid of newly devised techniques for the isolation of Z disc sheets from chicken skeletal muscle, we demonstrated that desmin actually surrounds each Z disc. On the basis of this information and other biochemical evidence, we proposed that desmin, in the form of 100 A intermediate filaments or some other polymeric form, functions to mechanically integrate all of the myofibrils in a muscle fiber at their Z lines. Thus, desmin and intermediate filaments may be the cytoplasm's molecular structure which is ultimately responsible for aligning myofibrils into their characteristic striated appearance. We have also discovered that desmin is a minor component of the filamentous cytoskeletons prepared by Triton X-100 extraction of chick embryonic fibroblasts and myoblasts. Interestingly

enough, we have discovered that the major component of the fibroblastic intermediate filaments (referred to here as F-IFP), which is distinguishable from desmin by molecular weight, isoelectric point and antigenic cross-reactivity, is a minor component of differentiated myotubes and isolated myofibrils. Using muscle cells differentiating in tissue culture as a model system, we plan to investigate a) the biosynthesis of F-IFP and desmin in fibroblasts and myoblasts, and during myoblast fusion into myotubes, and myotube differentiation into contracting muscle cells, b) the extent of homology of F-IFP and desmin by high resolution peptide map analysis and sequencing of putative homologous peptides, c) the immunofluorescent localization of desmin and F-IFP during muscle differentiation and myofibril assembly, and d) any putative interaction between desmin, F-IFP and actin during differentiation and myofibril assembly.

201. BIOCHEMISTRY OF CYTOSKELETAL PROTEINS

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The purpose of this research project is to study the structure and function of cytoskeletal proteins. Two major cytoskeletal proteins, desmin and vimentin, from hamster fibroblasts have been purified. Antisera against these proteins have been used to probe the organization of these proteins in fibroblasts and other tissues. The results so for indicate that both desmin and vimentin in normal cultured fibroblasts, BHK21/C13' are both present in the 10 nm filament system of the cell. Other cell systems have been studied for the presence of these two proteins. Platelets contain similar proteins and also platelet cytoskeletons possess a great deal of blood coagulation activity. The possible correlation between these two findings is being investigated.

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202. EFFECT OF MICROTUBULAR PROTEINS ON CELL SURFACES

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The abnormal growth pattern of transformed or neoplastic cells is associated with characteristic surface properties. For example, transformed cells are more agglutinable by various plant lectins than their normal counterparts. This seems to reflect a topographical heterogeneity of lectin binding sites on the cell surface. Recently we have obtained evidence that microtubular proteins (MT) are instrumental in effecting the heterogeneous distribution of such binding sites in virus transformed cells, and transport proteins in phagocytic cells. The main thrust of the study has three basic aims: to obtain further evidence that microtubular proteins are determinants of surface heterogeneity, to explore further the relationship of MT to surface phenomena, particularly agglutinability, and to characterize biochemically the association between membranes and MT. The first objective will be approached by direct isolation and biochemical analysis of membrane internalized during phagocytosis with and without treatment with the alkaloids colchicine or vinblastine which specifically bind to or disrupt microtubules, and the second, by studies of hemagglutination of normal and transformed cells with and without alkaloids as a function of the cell cycle, trypsinization, and other parameters, and the third, by cell fractionation and analysis of membrane-associated microtubular (colchicine binding) proteins.

These studies may serve as a basis for a new approach to the control of surface organization and hence of abnormal cell behavior.

203. CONTROL OF MICROTUBULE PROPERTIES AND FUNCTIONS

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A major obstacle to investigations of the control of microtubule properties and functions has been the lack of native tubulins derived from microtubules of defined characteristics and in quantities sufficient for biochemical studies. Outer doublet microtubules represent a model system for an investigation of this type, since the A-and B-subfibres differ not only from each other in morphology, function and chemical composition of their constituent tubulins, but also from brain microtubules. Until recently, it was not possible to exploit this system since tubulin solubilized from outer doublets was denatured. Assembly-competent tubulin has now been obtained from outer doublets and we propose to utilize this system to examine the control of microtubule properties and functions.

Our approach relies on methodology developed for brain microtubules which has been used to show that tubulin addition and loss from microtubules occurs at opposite ends of the microtubules, resulting in a flow of tubulin subunits through the microtubules. The importance of this concept is that it allows us to monitor factors influencing the tubulin flow rate and thereby gain insight into the control of microtubule properties and functions in vivo.

We propose to approach this problem in two stages. First, we plan to characterize the microtubules reassembled in vitro from outer doublet tubulin with respect to their opposite end assembly-disassembly parameters and exploit differences with vertebrate brain microtubules. Secondly, we plan to utilize the characterized assembly system as an assay method for examining the effect of chemically distinct tubulin preparations (derived from A- and B-subfibre microtubules) and axonemal proteins on the parameters of the assay system. In this way we hope to gain insight into the functional importance of tubulin chemistry for assembled microtubules, and the relevance of associated proteins for microtubule properties and function.

204. MICROTUBULE ASSEMBLY IN NORMAL AND TRANSFORMED CELLS

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The transformation of a normal cell to a cancer cell by viruses or carcinogens is often accompanied by dramatic changes in cell morphology. These changes seem to be mediated by changes in the cytoskeleton, particularly in the cytoplasmic microtubules and microfilaments. Very little is known about the molecular biology of these supramolecular structures, particularly about the cellular factors which regulate the time and location of their assembly. The objectives of this research are: (1) Characterization of proteins which positively regulate microtubule assembly, using immunofluorescent, electron-microscopic and biochemical methods. We are particularly interested in the possible role of phosphorylation of such tubulin assembly proteins by protein kinases in modulating their function. We will attempt to determine changes in their levels and state of activity (modification) during in vitro transformation of cultured fibroblasts. (2) Isolation and characterization of a new class of potential regulatory molecules (proteins, peptides): 'tubulin endorphins' which compete with colchicine for binding to tubulin and appear to be the physiological species which interacts with this site on microtubules. At least one of these compounds negatively regulates microtubule assembly. We will investigate where changes in their activity occur during transformation as a possible way of causing cytoskeletal disorganization. (3) Identification of transformation specific proteins which may alter microtubule assembly by binding to tubulin.

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205. TUBULIN STRUCTURE AND MICROTUBULE FORMATION AS SITES FOR PHARMACOLOGICAL ATTACK

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The rational development of new antineoplastic agents directed against tubulin, a protein critical for cell division, requires greater understanding of the interactions between the polypeptide subunits of tubulin and its two tightly bound guanine nucleotides. We have continued our studies on agents which stabilize this very unstable protein, and have found that several of these stabilizing agents also have the property of inducing tubulin to polymerize into microtubules in a reaction requiring no further addition except GTP. We have employed the most economical of these stabilizing agents, the amino acid glutamate, to develop a rapid, large-scale purification of tubulin. The purified protein is electrophoretically homogeneous and free of nucleoside diphosphate kinase and ATPase activities. The polymerization and GTPase activities of the purified tubulin in the glutamate-dependent system were examined. Finally, preliminary studies of the effect of analogues of GTP on the polymerization reaction were begun.

206. CHEDIAK-HIGASHI AND CANCER CELLS WITH SIMILAR DEFECTS

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Abnormal cell surface properties characterize leukocytes from several puzzling human diseases, including Chediak-Higashi syndrome, glutathione synthetase deficiency and Severe Combined Immune Deficiency. We have identified abnormal microtubule or microfilament properties that may explain the abnormal surface properties of these cells. We are now seeking the biochemical basis for the cytoskeletal-membrane defects. In particular we emphasize the role of purine compounds in microtubule assembly, and the role of glutathione metabolism in microtubule disassembly. We will also continue to define and catalogue cytoskeleton and membrane properties of various subsets of normal human lymphocytes.

207. NEOPLASTIC AND HYPERPLASTIC EPIDERMAL METABOLISM

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The overall objectives of this investigation are (a) to develop a molecular description and the mechanisms of control for inhibition of mitosis in the differentiated cell, for the biogenesis of the tonofilaments in the basal and lower spinous cells, for the formation of keratohyalin in the granular cells and for the loss of the nucleus and keratohyalin as the granular cell enters the stratum corneum, and (b) to define the molecular lesions responsible for the pathology in psoriasis, squamous cell and basal cell carcinomas and possibly other diseases of aberrant differentiation by developing and applying technology suitable for investigating abnormalities in the parameters elucidated in (a) to biopsy specimens of the pathological lesions. Methods to be applied include the biochemical isolation of the G1 inhibitor from differentiated cells and an evaluation of its status in hyperproliferative diseases, the use of antibodies to a tonofilament-protein to evaluate the protein's status in pathologies involving aberrant tonofilaments, an identification of changes in the carbohydrate of cell membranes during the differentiation of the keratinocyte and an elucidation of the role of histidine-rich protein in keratohyalin formation and the nature of the aberration responsible for absence of keratohyalin in parakeratotic conditions.

208. ANALYSIS OF MICROTUBULE ASSEMBLY MUTANTS IN CHLAMYDOMONAS

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The function of microtubule-associated proteins will be investigated by a genetic and biochemical analysis of the central pair of microtubules of Chlamydomonas reinhardtii. Microtubule-associated proteins have been identified by the requirement for their presence during in vitro polymerization of tubulin. I propose to isolate microtubule-associated proteins from wild-type Chlamydomonas by multiple rounds of polymerization and depolymerization of microtubules. Identification of mutations in genes coding for these microtubule-associated proteins will be accomplished by three complementary approaches. These include the analysis of peptide fragments, the genetic analysis of revertants, and the analysis of the in vitro polymerization ability of the mutant flagellar extracts. Once a gene and its polypeptide are found, the wild-type allele will be characterized by kinetic analysis to determine the effects of the protein on the rate and the extent of polymerization, by genetic analysis to look at other proteins which interact with it, and by ultrastructural and genetic analysis to find the intracellular location. The regulatory and structural role of these proteins in the assembly and stability of microtubules in vivo may be elucidated by this approach.

209. REGULATION OF CHLAMYDOMONAS FLAGELLAR PROTEIN ASSEMBLY

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I propose to determine the roles of synthesis, processing of proteins, and aggregation of proteins into subunits in controlling the assembly of a subcellular organelle, the flagellum, in Chlamydomonas. Deflagellation of Chlamydomonas induces a specific program of protein synthesis and regeneration of a new pair of flagella. I will identify precursors to the flagellar proteins which are synthesized in the cytoplasm in response to deflagellation, by their comigration with flagellar proteins on two-dimensional electrophoretic gels or their cross-reactivity with antibodies against flagellar proteins. The synthesis of flagellar proteins will be quantitated by scanning densitometry of gel autoradiograms to determine whether the proteins are assembled into the flagellum in the same relative amounts as they are synthesized in the cytoplasm. The kinetics of transport of flagellar proteins from their site of synthesis in the cytoplasm during a radioactive pulse label to their sites of assembly in the flagellum during a nonradioactive chase will be determined. Finally, I will attempt to isolate aggregates of flagellar proteins from the cytoplasm of regenerating cells by gradient centrifugation and affinity chromatography. Analysis of normal regenerating cells will be paralleled by analysis of regenerating flagellar mutants, and analysis of drug-blocked flagellar growth and flagellar resorption.

210. A BIOCHEMICAL ANALYSIS OF ASPERGILLUS MITOTIC MUTANTS

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The proposed research seeks to study the biochemistry of mitosis by means of a detailed analysis of the temperature sensitive, mitotic mutants of Aspergillus nidulans. In particular, it will be determined if a unique set of tubulins comprises the microtubules of the mitotic apparatus. If this be the case, experiments will be conducted to determine if these tubulins are the products of mitotic tubulin genes or are post-translational modifications of a common tubulin gene product. In addition, experiments will be undertaken to detect if either a dynein-like ATPase or actin and myosin is associated with the mitotic apparatus. If present, then the mitotic mutants will be analyzed to determine if any of these proteins is responsible for generating mitotic movements. By correlating biochemical data with genetic information and ultrastructural analysis of the mutants, it will be possible to determine at what time during mitosis specific proteins are required. Moreover, a study of extragenic suppressor revertants of the mitotic mutants will provide information about the interaction of various mitotic proteins. This combined approach will help elucidate the mechanisms of mitotic movements.

211. NERVE CELL MICROTUBULE-INTERMEDIATE FILAMENT INTERACTIONS

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Interactions of microtubules with intermediate filaments have been observed with the electron microscope in many types of cells, and particularly in nerve (e.g., B. Bertolini et al., 1970, J. Ultrastruct. Res. 33:173). By isolating intact microtubule fila-

ment complexes and by reconstructing the complexes in vitro from microtubules and intermediate filaments, (a) isolated intact and (b) reassembled from their subunits, using proteins from cultured nerve cells and from brain, the specific protein subunits responsible for the interactions will be identified. These studies will require identification of 'backbone' and associated proteins of intermediate filaments, and determination of conditions for disassembly and reassembly of intermediate filaments. The microtubule associated proteins that have been identified by their co-purification with tubulin will also be compared with those present on microtubules isolated intact. These studies are directed toward understanding how neurons transport metabolites and organelles over fairly long distances, and toward answering the more general questions of cytoplasmic motility which are thought to involve these filamentous structures.

212. MICROTUBULIN REGULATION - CONTROL OF SYNTHESIS OF TUBULIN

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No summary has been provided to the Smithsonian Science Information Exchange.

B. BIOCHEMISTRY OF OTHER CYTOPLASMIC PROTEINS

213. ENZYME ORGANIZATION OF THE CYTOPLASM, WITH SPECIAL REFERENCE TO THE LOCALIZATION AND FUNCTION OF ELECTRON- AND ENERGY-TRANSFER ENZYMES

Ernster, L., Nelson, B. D., Rydstrom, J., Stockholms Universitet, Arrhenius Lab., Dept. of Biochemistry, *116 Drottninggatan, Box 6801, S10691 Stockholm, Sweden*

OBJECTIVE: The research carried out under this program concerns the structure and function of enzymes involved in mitochondrial electron transport and oxidative phosphorylation. Present efforts are focused on three enzymes: the cytochrome b-c1 complex, nicotinamide nucleotide trans-hydrogenase, and the ATP-synthesizing enzyme (ATPase).

APPROACH: All three enzymes have been studied both in the isolated, purified form and in the membrane-bound form as they occur in submitochondrial particles and intact mitochondria. Reconstitution of the three enzymes, alone or in combination, has also been carried out, using artificial phospholipid vesicles. The parameters studied involved various structural and catalytic properties of the three enzymes as well as their ability to generate a proton gradient across the membrane.

PROGRESS: The cytochrome b-c1 and ATPase complexes have been characterized with respect to their subunit composition, and attempts have been made to identify the function of the various subunits. A reconstitution of the ATPase system from isolated subunits has also been initiated. The trans-hydrogenase has been studied primarily from the metabolic point of view, using reconstituted liposomes, intact mitochondria and cultured rat heart cells.

214. CROSS-MODAL MATCHING IN HUMAN NEONATES

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Dr. Meltzoff has been conducting studies with our support over this past year on the capacity for imitation in very early infancy. He discovered an interesting phenomenon in the course of that work, namely, that 28-day-old infants can visually recognize a shape they had explored tactually. Such coordination between the senses, generally known as cross-modal matching, is an important aspect of perception and cognition, and its discovery among new-borns could alter current theories about cognition in infancy. Dr. Meltzoff's current research will tell him if the infants' cross-model abilities change in any significant ways through the fifth month of life. The solid base of data from this study will aid future explorations of this phenomenon.

Dr. Meltzoff will conduct three more studies over the next year. The first probes the nature of the representational capacities, or early memory systems, that permit an infant to do cross-modal matching. The experiment will consist of allowing infants to explore a tactual object orally, removing it, and then presenting visual objects for recognition after variable amounts of time. The second and third studies will vary certain features of the objects to be matched, such as shape, general form, and whether they are two or three dimensional, in an attempt to assess the limits of this ability, and to compare this phenomenon with some related developmental research done in the past.

Besides being theoretically important, Meltzoff's work also has some practical applications. Deficiencies in cross-modal matching are known to be related to reading problems, psychiatric disturbances, and other personality and cognitive traits. The proposed research could be helpful in the development of tests to identify, at a very early age, children at risk for abnormal development.

215. DEVELOPMENTALLY REGULATED CYCLIC AMP-BINDING PROTEINS

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Cyclic AMP is thought to be required for the expression of most or all developmental gene products. In attempting to gain some understanding of the mechanism(s) by which cyclic AMP exerts its effect on gene expression I have detected five developmentally regulated cyclic AMP-binding proteins by gel electrophoresis and by gel filtration. The kinetics of appearance of these cyclic AMP-binding proteins coincide with the pattern of accumulation of cellular constituents known to be modulated by cyclic AMP during specific stages of development. These results are consistent with the idea that the cyclic AMP-binding proteins play a role in controlling the expression of stage specific genes. Further, some of these cyclic AMP-binding proteins, in association with the spore and stalk factors, may regulate the expression of cell-type-specific genes.

216. STUDY OF A LIVER HELIX DESTABILIZING PROTEIN IN VIVO

Patel, G. L., University of Georgia, Franklin College of Arts & Sciences, Dept. of Zoology, *Athens, Georgia*, 30601, United States of America

A helix destabilizing protein has been purified from rat liver. This project concerns identification of its role in replication and/or transcription, and elucidation of the mechanism(s) underlying regulation of its activity. Hepatoma tissue culture cells are being used to investigate the synthesis, turnover, post-translational modifications, and cyto-nuclear translocation of this protein during cell cycle and following dexamethasone induced transcription. Temporal correlation of these data with levels of replication and transcription in vivo will indicate its involvement in these processes. Supportive evidence is also being obtained from effects of this protein on DNA and RNA polymerase reactions in vitro. Data obtained thus far suggest a transcriptional role for this helix destabilizing protein.

217. MOLECULAR AND CELLULAR STUDY OF CANCER PROCOAGULANT A

Gordon, S. G., University of Colorado, Health Sciences Center, School of Medicine, Dept. of Medicine, *4200 E. 9th Ave., Denver, Colorado*, 80262, United States of America

A unique coagulation enzyme has been isolated from malignant tissue, purified to homogeneity and partially characterized. This enzyme, cancer procoagulant, has been identified as a cysteine protease that initiates coagulation by directly activating factor X in the coagulation cascade. Studies of the enzymatic activity of cancer procoagulant in cell suspensions have demonstrated that it is present in the B16 mouse melanoma cells that were grown in defined medium (in the absence of fetal bovine serum) for 90 days, suggesting that the enzyme is not derived from serum components or extracellular substances but rather synthesized by the malignant cell. In addition, cell suspensions of the HT-29 human colon carcinoma cells and SV-40 transformed hamster fibroblasts contained the same procoagulant activity. In contrast normal hamster fibroblasts contained a procoagulant that was not DFP sensitive and appeared to be tissue factor.

We are continuing to study the physical, chemical and enzymatic properties of this enzyme. In addition, we are studying the role of the enzyme in the malignant and metastatic

process so as to understand whether the enzyme directly or indirectly facilitates the malignant process.

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218. REGULATION OF THE BIO-SYNTHESIS AND SECRETION OF OPIOID PEPTIDES

Sabol, S. L., Dandekar, S., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Heart Lung & Blood Inst., Molecular Biology Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

Methionine-enkephalin and leucine-enkephalin are endogenous pentapeptides that possess opiate activity. The bovine adrenal medulla, which synthesizes and secretes enkephalins, has been chosen for the investigation of the biosynthesis of the enkephalins. Messenger RNA (mRNA) from adrenal medulla has been isolated and used as a template for the cell-free synthesis of enkephalin precursor protein in the wheat germ and rabbit reticulocyte protein-synthesis systems. Specific proteolytic digestion of the adrenal mRNA-dependent proteins yields methionine-enkephalin. The enkephalin precursor protein and enkephalin mRNA are currently being characterized with the goal of understanding the regulation of synthesis of enkephalins, which are thought to be important in pain perception and behavior.

BIBLIOGRAPHIC REFERENCES: Sabol, S.L., Storage and secretion of beta-endorphin and related peptides by mouse pituitary tumor cells: regulation by glucocorticoids. Arch. Biochem. Biophys. 203, 37-48, (1980).

219. THE MECHANISM OF ACTION OF THYMIDYLATE SYNTHETASE

Dunlap, R. B., Hopper, W. E., Freedlander, R. S., Ahmed, F., McAleese, D. L., University of South Carolina, School of Science & Mathematics, Dept. of Chemistry, *Admin. Bldg., Room 115, Columbia, South Carolina,* 29208, United States of America

This project is conccerned with the mechanism of action of thymidylate synthetase (deoxyuridylate plus 5,10-methylenetetrahydrofolate yields thymidylate plus 7,8-dihydrofolate) from amethopterin-resistant Lactobacillus casei. The problems to be investigated include (a) characterization of pseudo ternary complexes of thymidylate synthetase, (b) equilibrium gel filtration studies of binary enzyme complexes, (c) active site crosslinking studies, (d) modification of carboxyl groups with water-soluble carbodiimides, (e) characterization of enzyme-Cibacron Blue interactions.

BIBLIOGRAPHIC REFERENCES: J.W. Reinsch, L.L. Smith, and R.B. Dunlap, Denaturation of Thymidylate Synthetase from Amethopterin-Resistant Lactobacillus casei, Cancer. Biochem. Biophys. 3, 57-64 (1979). C.A. Lewis, Jr., P.D. Ellis and R.B. Dunlap, Fluorine-19 Nuclear Magnetic Resonance Investigation of the Noncovalent and Covalent Binary Complexes of 5-Fluorodeoxyuridylate and Lactobaccilus casei Thymidylate Synthetase, Biochemistry 19, 116-123 (1980).

220. CYTOPLASMIC PHOSPHOPROTEINS AND THE REGULATION OF CELL GROWTH

Kletzien, R. F., West Virginia University, School of Medicine, Dept. of Biochemistry, *Medical Ctr. Dr., Morgantown, West Virginia,* 26506, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

221. ENZYME CHANGES ASSOCIATED WITH MALIGNANCY

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This is part of a broader project. A summary of this subproject is not available.

C. BIOCHEMISTRY OF COLLAGEN

222. COLLAGEN AND ITS RELATIONSHIP TO TUMORS

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OBJECTIVES: To establish the role and relationship of extracellular matrix, particularly collagen, to tumor growth and metastasis.

RESEARCH PLAN: 1) The amount and type of collagen produced by oncogenic and metastatic cells are being compared to non-oncogenic cells. 2) The cell attachment of these cells to different types of collagen and connective tissue substratum are being investigated. 3) The collagen type and distribution in tumors and capsules formed by oncogenic cell lines are being studied in order to determine if the oncogenic cell or the host fibroblasts are responsible for tumor stroma production.

METHODOLOGY: Cells are grown in tissue culture and pulsed with radioactive amino acids. The collagen produced by these cultures or in tumors are extracted and purified by salt precipitation, column chromatography, and gel electrophoresis. The types of collagen are finally determined by cyanogen bromide peptide analysis. The relative collagen production is measured by purified collagenase assays or hydroxyproline production.

FINDINGS: Relative collagen production decreases in all chemically transformed oncogenic cell lines. Several cell lines, both fibroblastic and epithelial have been tested. In the case of N-acetoxy-acetylaminofluorene treated rat liver epithelial cells, the type of collagen produced is altered. The reason for the change in collagen type is being investigated. Preliminary studies show that the tumors produced by these cells contain the altered collagen molecule.

223. COLLAGEN AND ITS RELATIONSHIP TO TUMORS

Smith, B. D., Katz, M., Boston University, School of Medicine, Dept. of Biochemistry, *80 E. Concord St., Boston, Massachusetts,* 02215, United States of America

The goal of this project is to establish the role and relationship of collagen matrix to tumor growth and metastasis. The first objective is to establish the structure, organization, function, type and metabolism of collagen in malignantly transformed cells and in tumors. Currently the collagen metabolism of chemically transformed cells is being compared to that of their parent cell lines. The collagen distribution and type in tumors produced by these cell lines is being determined in order to establish the origin of connective tissue in tumors, that is, whether collagen is synthesized as a result of host response to tumor growth and/or a result of oncogenic cell synthesis. Finally, studies are being initiated to determine whether the cell matrix interactions are important in metastasis.

BIBLIOGRAPHIC REFERENCES: Yue, B.Y.J.T., Baum, J.L. and Smith, B.D. Collagen Synthesis By Cultures of Stromal Cells from Normal Human and Keratoconus Corneas, Biochem. Biophys. Res. Commun., 86:465-472 (1979).

224. COLLAGEN BIOSYNTHESIS BY CULTURED HUMAN FIBROBLASTS

Uitto, J. J., Ryhanen, L. J., Oikarinen, A. I., Tan, E. M., University of California, Los Angeles Campus, Los Angeles County Harbor General Hospital, *1000 W. Carson St., Torrance, California,* 90509, United States of America

In this grant application we are proposing extensive studies of the metabolism of collagen and its biosynthetic precursor, procollagen, by cultured human skin fibroblasts in control and disease states. The major goal of this research is to exactly define the molecular defects which may occur in the biochemistry of collagen in diseases affecting the connective tissues of skin and other organs. The major emphasis will be on diseases in which previous investigations by us and others have revealed connective tissue abnormalities, but in which the exact nature and the exact extent of collagen aberrations are currently unknown. Such diseases include psoriasis, benign and malignant skin tumors, scleroderma, the Ehlers-Danlos syndrome, and osteogenesis imperfecta. For comparison, parallel studies will be performed employing control fibroblasts from subjects without connective tissue abnormalities. The parameters to be studied

include rate of procollagen synthesis by the culture fibroblast, as well as isolation, identification, and characterization of the genetically distinct procollagen. In order to understand changes in collagen metabolism in the fibroblast cultures in diseases, additional studies will be performed on the structure and metabolism of genetically distinct procollagens synthesized by normal human skin fibroblasts. Specifically, the procollagens will be isolated in quantitative amounts in order to allow further chemical characterization. The purification of the procollagens will also enable us to develop radioimmunoassays, based on type-specific antibodies, which will then be utilized in studies of collagen formation in diseases. The antibodies are also employed in investigations concerning the control mechanisms involved in the biosynthesis of procollagen as well as in identification of products in a cell-free translation system to be developed for assay of procollagen messenger-RNA.

225. STUDY OF THE FACTORS REGULATING THE SYNTHESIS OF COLLAGEN IN NORMAL AND TRANSFORMED CELLS

Peterkofsky, B., Bateman, J., Chojkier, M., Chauhan, U., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Biosynthesis Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

We are studying the role of ascorbic acid and neoplastic transformation in regulating collagen metabolism in animal cells. Deficiency of ascorbate in guinea pigs leads to decreased proline hydroxylation and defective collagen synthesis. In contrast, stationary cultures of L-929 cells do not require ascorbate for proline hydroxylation and contain a substance in the microsomal membrane which can effectively hydroxylate proline during procollagen synthesis in the rough endoplasmic reticulum.

BIBLIOGRAPHIC REFERENCES: Peterkofsky, B., Kalwinsky, D., and Assad, R.: A substance in L-929 cell extracts which replaces the ascorbate requirement for prolyl hydroxylase in a tritium release assay for reducing cofactor: correlation of its concentration with the extent of ascorbate-independent proline hydroxylation and the level of prolyl hydroxylase activity in these cells. Arch. Biochem. Biophys. 199: 362-373, 1980.

226. INTERACTION OF NORMAL AND MALIGNANT CELLS WITH COLLAGEN

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The phenomenon of cell movement is a major property of both embryological development and of metastatic growth, and is thought to involve recognition of the substrate by the migrating cells. Substrates of glycosaminoglycans and glycolipids are altered by migrating cells in a specific manner which indicates that the recognition system may involve a glycosyltransferase on the cell surface. This project is designed to test the possibility that surface glycosyltransferases are causally involved in cell migration, using the common extracellular component, collagen, as the substrate. The interaction of normal, malignant, and embryonic cells with collagen will be tested in three ways. First, both substrates of the enzyme (collagen and the appropriate UDP-sugar) will be provided exogenously to 3T3 cells or their transformed counterparts, 3T12 cells. Transfer of labeled sugar to collagen will be monitored, and the specificity of the reaction will be tested. Next, the UDP-sugar will be deleted to test the ability of the cells to provide their own sugar donor. Third, the specific glycosyltransferase inhibitor, UDP-dialdehyde, will be tested to see if it inhibits migration to the same extent as it inhibits glycosylation.

If the data from the 3T3 and 3T12 cells allow firm conclusions, neural crest cells will be tested in the same way since these cells undergo extensive migrations in the embryo and they also exhibit high levels of several glycosyltransferases. Migration-deficient mutants of neural crest cells will be tested to determine if any are deficient in ability to glycosylate collagen.

Little is known about the factors involved in the initiation or cessation of cell movement, except that these processes occur during abnormal, metastatic growth and during normal embryological development. This proposal is intended to collect basic information about cell migration, as well as to address a specific area of cancer cell behavior, that of metastasis.

227. BASEMENT MEMBRANE COLLAGEN DEGRADATION AND ITS ROLE IN TUMOR INVASION AND METASTASES

Liotta, L., Garbisa, S., Foltz, C., Lee, C., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Pathophysiology, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

There are at least five genetically distinct collagen types whose degradation may be controlled independently. The initial step of collagen degradation is performed by collagenase. We and others have found that type IV basement membrane collagen is not degraded by human skin collagenase suggesting that a separate collagenase may degrade type IV collagen. A collagenase which preferentially degrades type IV collagen has been derived from a metastasis of the T241 fibrosarcoma. This collagenase has been purified 1000-fold and its cleavage products have been partially characterized. We are further studying the secretion rate of this enzyme by a wide variety of cell types both normal and malignant.

BIBLIOGRAPHIC REFERENCES: Murray, J.C., Liotta, L.A., Rennard, S.I., and Martin, G.R. Adhesion characteristics of metastatic and nonmetastatic tumor cells in vitro. Cancer Res. 40: 347-351, 1980. Liotta, L.A., Tryggvason, Kr., Garbisa, S., Foltz, C., and Shafie, S. Metastatic propensity correlates with tumor cell degradation of basement mebrane collagen. Nature 284: 67-68, 1980.

228. BASEMENT MEMBRANE COLLAGEN SYNTHESIS AND DEGRADATION IN THE NORMAL AND NEOPLASTIC MAMMARY GLAND

Liotta, L. A., Garbisa, S., Kidwell, W., Lee, C., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Cycle Regulation Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

We have purified and characterized mammary basement membrane type IV collagen, studied its synthesis and turnover in normal and neoplastic mammary gland. We have further purified collagens I, III and IV from human mammary gland and studied their relative amounts in mammary disease states.

BIBLIOGRAPHIC REFERENCES: Kleinman, H.K., Hewitt, A.T., Murray, J.C., Liotta, L.A., Rennard, S.I., Pennypacker, J.P., McGoodwin, E.B., Martin, G.R. and Fishman, P.H. Cellular and metabolic specificity in the interaction of adhesion proteins with collagen and with cells. J. Supramol. Struct. II: 69-78, 1979. Wicha, M.S., Liotta, L.A., Garbisa, S., and Kidwell, W.R. Basement membrane collagen requirements for attachment and growth of mammary epithelium. Exp. Cell Res. 124: 181-190, 1979.

229. SUBSTRATUM REQUIREMENTS FOR NORMAL AND NEOPLASTIC MAMMARY EPITHELIAL CELL GROWTH

Kidwell, W., Liotta, L. A., Salomon, D., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Cell Cycle Regulation Section, *9000 Rockville Pike, Bethesda, Maryland,* 20205, United States of America

In vivo the mammary epithelium rests upon a basement membrane containing type IV collagen. This collagen substratum is made primarily by a myoepithelial-like cell and appears to be essential for the growth and survival of the epithelial cell population since the epithelial cells degenerate and die in vivo and in vitro if collagen deposition is prevented following a proliferative stimulus. In vitro studies suggest that mammary growth control may be regulated by the action of basement membrane deposition by the myoepithelial cells. Involution is apparently triggered by the breakdown of preformed basement membrane catalyzed by a specific collagenase produced by the epithelium upon withdrawal of hormonal support. Some primary tumors appear to retain a requirement for basement membrane collagen synthesis since they regress when the host is administered cis-hydroxyproline to block collagen deposition.

BIBLIOGRAPHIC REFERENCES: Wicha, M.S., Liotta, L.A., Garbisa, S. and Kidwell, W.R. Basement membrane collagen requirements for attachment and growt.. of mammary epi-

thelium. Exp. Cell Res. 124: 181-190, 1979. Liotta, L.A., Wicha, M.S., Rennard, S., Foidart, J.M. and Kidwell, W.R. Hormonal requirements for basement membrane collagen deposition by cultured rat mammary epithelium. Lab. Invest. 41: 511, 1979.

230. KELOIDS - AN IN VITRO MODEL OF TUMOR GROWTH REGULATION

Russell, J. D., Russell, S. B., Trupin, K. M., Meharry Medical College, School of Graduate Studies & Research, Dept. of Microbiology, *1005 18th Ave. N., Nashville, Tennessee*, 37208, United States of America

Keloids are dermal tumors which occur in response to trauma. A hereditary predisposition to Keloid formation occurs with high frequency in black populations. In addition to overproliferation of fibroblasts this tumor growth involves abnormal collagen metabolism.

The goals of these studies are to identify the primary gene defect causing this condition, its mode of action, and those environmental factors which alter its expression. The strategy used in this investigation is to search for patterns of differences in cell culture between fibroblasts derived from keloids and from normal scars and skin. We have found that cultured fibroblasts isolated from normal and keloid tissue are similar in regard to both growth characteristics and rate of collagen production; however, the addition of physiological levels of hydrocortisone to the culture media results in significant differences between these cell types in both their growth characteristics and rate of collagen production, suggesting an abnormality in the regulation of both collagen metabolism and growth in keloid cells.

The studies described here are aimed at elucidating the regulatory mechanism functioning abnormally in keloids and the relationship between the abnormal phenotypes observed, namely collagen metabolism and cell growth, by determining (1) the genetic and epigenetic contributions to these phenotypes; (2) whether the phenotypes are cell limited and the dominance relationships between them; (3) the mechanisms of the response to hydrocortisone; (4) the ability of other environmental variables to elicit or prevent differences between normal and keloid fibroblasts.

Results to date suggest coordinate regulation of several steps in collagen metabolism by hydrocortisone and that the abnormality in keloid cells affects a regulatory process common to several of these steps.

231. COMPARATIVE BIOCHEMISTRY OF CARTILAGE TUMORS

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The objectives of this research are to compare the fine structure and modulation of synthesis of proteoglycans in normal cartilage, human benign and malignant cartilaginous tumors, and a model system - the Swarm rat chondrosarcoma. There will be an emphasis on the differences between aggregating and nonaggregating proteoglycans, the proteoglycan link-protein relationship, and the occurrence of keratan sulfate or 'keratan sulfate-like' glycopeptides. The methods will involve chemical characterization of isolated proteoglycan fractions and analysis of isotopically labeled materials from cultured cells. Proteoglycans will be extracted from the tissue with guanidinium chloride in the presence of protease inhibitors and purified in cesium chloride gradients under associative conditions. Fractions will be assayed physically, biochemically, and immunologically. Selected fractions will be degraded (either chemically or enzymatically), fractionated, and reassayed. Cells will be grown, pulsed with precursors (H3-GlcN and C14-serine), and the labeled products analyzed to determine the rate of synthesis and nature of the products produced. The noncollagen, nonproteoglycan matrix molecules will also be examined electrophoretically and detected either chemically or by autoradiography.

232. ISOLATION AND CHARACTERIZATION OF ANTIBODIES TO COLLAGEN/PROCOLLAGEN

Goldberg, B. D., New York University, School of Medicine, Dept. of Pathology, *100 Washington Sq. E., New York, New York*, 10003, United States of America

OBJECTIVES: To produce high titer antibodies to the carboxyl-terminal propeptides of human type I procollagen. To produce high titer antibodies to the endopeptidase which specifically cleaves the carboxyl-terminal propeptides of human type I procollagen. To produce the above antibodies in sufficient quantities so that they may be made available to other investigators.

233. ISOLATION AND CHARACTERIZATION OF ANTIBODIES TO COLLAGEN, PROCOLLAGEN

Furthmayer, H., Yale University, School of Medicine, Dept. of Pathology, *333 Cedar St., New Haven, Connecticut*, 06510, United States of America

OBJECTIVE: Prepare and isolate immunochemically defined antibody reagents to collagen of types I, II, III, IV (basement membrane), AB (fetal collagen), and procollagen of types I and III which react specifically with human proteins. Prepare similar reagents specific for mouse collagen of types I, III, and IV (basement membrane).

D. BIOCHEMISTRY OF PROTEINS HAVING CLINICAL SIGNIFICANCE

1. BIOCHEMISTRY OF DIAGNOSTICALLY IMPORTANT ECTOPIC PROTEINS

234. CHEMICAL AND IMMUNOLOGICAL STUDIES ON CARCINOEMBRYONIC ANTIGEN -CEA

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OBJECTIVE: To improve the disease-related specificity of the CEA-test and to understand the function of CEA and the molecular mechanisms responsible for its appearence in the tumor cell.

APPROACH: Isolation and characterization of CEA-related normal adult tissue components. Quantitation of such substances in sera from patients with cancer and other diseases. Structural studies on the carbohydrate and peptide moieties of CEA. Preparation of monoclonal antibodies to CEA. Studies on the expression of CEA and related normal antigens in different human carcinoma lines.

PROGRESS: We recently isolated a new CEA-related adult tissue component from bile and serum. This glycoprotein, biliary glycoprotein I (BGPI), has a molecular weight of approximately 85,000 and is present at a concentration of 0.5 plus or minus 0.3 mg/l (mean plus or minus 2SD) in healthy individuals. Most patients with liver disease (chronic hepatitis, alcoholic cirrhosis, primary biliary cirrhosis, hepatitis A and B, CMV hepatitis, obstructive jaundice) have elevated levels; up to 5-10 the upper reference limit. The great majority of patients with gastrointestinal disease (ulcerative colitis, Crohn's disease and other) have normal values. Patients with gastrointestinal cancer generally have elevated or slightly elevated levels. In patients with liver disease, serum BGPI is positively correlated to gamma-glutamyltranspeptidase (GT). Serum BGPI is a parameter of cholestasis of similar use as serum-GT.

Spleen cells from BALB/c mice immunized with highly purified CEA were fused with the myeloma line Sp2/0 at a ratio of 1:1. Out of 51 antibody-producing colonies, 8 were selected and cloned twice over a period of 4 months of in vitro culture. Monoclonal anti-CEA antibodies with 5 different specifications were obtained. All were IgG1, but showed different isoelectric points. One antibody was equally reactive with CEA from four individual GI tumors but nonreactive with nonspecific cross-reactive antigen (NCA).

235. PREALBUMIN SUBFRACTIONS IN BRAIN TUMORS

Uki, J., Takeda, F., Saitama Cancer Center, Neurosurgical Clinic, *818 Komuro, Ina-Machi, Kitaadachi-Gun, Saitama-Ken 362, Japan*

OBJECTIVE: By the advancement of protein chemistry, oncofetal antigens have become the major problem in immunodiagnosis for cancer patients. In the central nervous system, acidic or basic 'brain specific proteins' are currently studied regarding their biological functions and their clinical applications. Our attempt is to determine and purify the oncofetal 'brain specific protein' or brain tumor specific protein in the mixed acidic proteins, prealbumin (PA) fraction, in order to make them clinically useful.

APPROACH: Step 1: Biochemical qualification and quantification of PA fraction and its subfractions are analyzed by 7.5 percent DISC-PAG electrophoresis and densitometric calculation; comparison was made between fetal-adult brain, brain tumors, other organs, and body fluid from sera, CSF, and cystic brain tumor. Step 2: Each PA subfraction is immunochemically identified, whether it is a known antigen or not. Step 3: Some of the PA subfractions with the oncofetal 'brain specific proteins' or brain tumor specific protein are purified and the antibody prepared for furthering immunochemical studies. Step 4: The specific antigens thus obtained are investigated for the clinical immunodiagnostic use.

PROGRESS: PA subfractions were analyzed biochemically in 226 cases, in which tissue PA was mainly composed of 5 fractions, PA-I through PA-V from the anode to the cathode, and each fraction was further subfractionated into the 2-3 bands, while fluid PA showed only one peak corresponding to the tissue PA-II'. Both brain tumor and fetal brain contrained higher PA-III and lower PA-IV in concentration than did adult brain. Antibody to the whole PA fraction obtained from neurinoma contained neurinoma specific protein. We have been currently undertaking the isolation of the PA-III and the production of its antibody in gliomas, meningiomas, and neurinomas.

236. THE BIOLOGICAL ROLE OF ALPHA-FOETOPROTEIN

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OBJECTIVE: The biological parameters which accompany the appearance of alpha-foetoprotein in serum have been studied by many authors; the production of alpha-foetoprotein by transitional cells is related to cellular proliferation. The study of the role of this protein in tumorigenesis is the main aim of this project.

APPROACH: The role of AFP is being studied using models at the cellular level; cell fusion using PEG will be used to elucidate whether the production of AFP by neoplastic cells can be inhibited by fusion with normal hepatocytes. The production of AFP will be compared with other neoplastic properties. The hypothesis that AFP is a carrier of a growth factor will be tested using the hepatocyte cell culture and by binding studies in vitro.

The relationship between the appearance of AFP and another marker of hepatoma, gamma-glutamyl transpeptidase, as well as the phenotype expression of GGT in neoplasia, will be studied.

PROGRESS: Quantitative modifications of GGT were observed during chemical carcinogenesis but no GGT isoenzyme difference could be observed after tumor induction with 3'-Me-DAB as indicated by ConA-Sepharose chromatography, electrophoresis and Km determination. GGT elevation in our conditions seems to be related to a detoxication process rather than to derepression.

237. EMBRYONIC, PLACENTAL AND ECTOPIC PROTEINS IN HUMAN AND EXPERIMENTAL TUMORS

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Using immunohistochemical and radioimmunoassay techniques, we have demonstrated the presence of chorionic gonadotropin (CG), or an antigenically cross-reacting substance, in various experimental rat tumors. We feel that it would be important to establish whether the polypeptide observed is biologically active and whether it is glycosylated and therefore of the 'placental' type, rendering its presence in tumors ectopic. We propose to isolate and purify CG from a variety of tumor models, both hepatomas and breast cancinomas, using extracts of placenta, liver and other tissues as controls. The CG content of these materials will be assayed by radioimmunoassay (with a specific antiserum to the beta-subunit moiety of CG) and by bioassay, measuring testosterone production in rat Leydig cell cultures. The molecular size of the various CG-like peptides will be determined by chromatography. By using affinity chromatography on Sepharose-concanavalin A columns, we hope to establish whether the CG resembles the normal placental polypeptide. If this is the case, we have a re-expression of a very early ontologic event; on the other hand, if the CG is non-glycosylated, we may be observing the increased synthesis of a ubiquituous 'pro' polypeptide or a substance which is rapidly desialyted and has therefore a very short biological half-life. Tumor CG could influence the biological behavior in terms of growth, local invasiveness and metastatic potential of the model.

238. EVALUATION OF HUMAN EMBRYONIC PREALBUMIN AS A TUMOR MARKER

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OBJECTIVE: The aim of this study is to evaluate the role of the recently identified fetal plasma protein, human embryonic prealbumin (EPA) as a marker for the detection and monitoring of patients with malignant connective tissue tumors.

APPROACH: Following the isolation and purification of EPA a sensitive radioimmunoassay for the measurement of EPA in serum and tissue homogenates will be developed.

By screening selected pre- and postoperative sera from patients with malignant mesodermal tumors for EPA it will be possible to determine the usefulness of EPA as a marker for the early detection of tumor recurrence.

239. ECTOPIC PRODUCTION OF PLACENTAL PROTEINS BY NON-TROPHOBLASTIC TUMORS

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OBJECTIVE: Demonstration of ectopic production of placental proteins by non-trophoblastic tumors.

APPROACH: Immunohistochemical demonstration of placental proteins in tissue sections. Radioimmunoassay of serum from tumor patients and of homogenates of tumors.

PROGRESS: Immunohistochemical studies suggest PP6 not useful. Radioimmunoassay developed for PP5. Large range of sera from breast cancer patients, and homogenates of excised tumors screened for SP1 and PP5. Attempts to purify SP1 from large tumors (liver secondaries of bowel primary) unsuccessful because SP1 content low.

240. ECTOPIC PROTEIN AND SUBUNIT PRODUCTION BY TUMORS

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We continue to study placental proteins as cancer markers. The pregnancy-specific beta1 glycoprotein (SP1) is produced by normal human fibroblasts but does not appear to be a useful marker in cases of fibrosarcoma, osteogenic sarcoma, glioma or breast carcinoma. Placental protein 5 is also made by human fibroblasts in vitro but its regulation by the cell appears to be different from that of SP1. Chemical modification of SP1 is being explored in an attempt to understand its unusual CD spectrum. Placental lactogen (PL) is made by different cells of a testicular choriocarcinoma than is alpha subunit. Large forms of PL have been detected in serum and placenta at term, even when blood and tissue have been rapidly processed. Analysis of oligosaccharides released from CG-alpha by trifluoroacetolysis has revealed heterogeneity unrecognized in current structural formulas of this glycoprotein. HeLa cells make alpha subunit in serum-free media with growth factor supplementation, at rates up to 80 percent of those in standard medium containing fetal calf serum.

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241. THE PLACENTA SPECIFIC PROTEIN, SP1, IN CANCER

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The placenta specific protein, SP1, is an oncoplacental protein: it is produced in the placenta and in certain tumors. It is present in the serum and urine of pregnant women and in the serum of patients with trophoblastic tumors. Evidence exists for the production of SP1 in tumors other than trophoblastic.

We intend to study the basic molecular characteristics of SP1 and the expression of SP1 in the normal state and in malignant diseases: what types of tumors produce SP1, what are the levels of SP1 in the serum and urine of tumor patients, and what are the normal levels of SP1. Quantitation of SP1 is done by radioimmunoassay.

An enzyme-linked immunosorbent assay (ELISA) will be developed for SP1. Increased sensitivity is achieved by using a fluorigenic enzyme substrate instead of a colorigenic substrate.

For possible future specific immunotherapy of SP1 producing tumors, a nonprimate animal model is necessary. We will attempt to identify SP1 in laboratory animals by immunological techniques.

242. THE PLACENTA SPECIFIC PROTEIN, SP1, IN CANCER

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We are studying the molecular characteristics of the oncoplacental protein SP1 and its expression in some in vivo and in vitro conditions. SP1 is found in the serum of pregnant women, of most patients with gestational trophoblastic tumors, and of some patients with germ cell tumors. SP1 can neither be found in the circulation of nonpregnant healthy individuals nor of patients with cancer of the digestive tract, breast cancer, melanoma and sarcoma. SP1 is consequently not a general tumor marker, but assays for SP1 may be useful in monitoring patients with tumors of the reproductive system.

In vitro, all skin fibroblasts from normal adults have been found to produce SP1. In contrast, most human tumor cell lines tested, including some of trophoblastic origin, did not produce detectable amounts of SP1. Only glioma was found to produce SP1 in vitro, and the production of SP1 was retained when the tumor was growing in nude mice. The apparent discrepancy between the in vivo and in vitro expression of SP1 should provide interesting insights into the regulation of expression of oncodevelopmental proteins once its molecular basis is understood.

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2. BIOCHEMISTRY OF DIAGNOSTICALLY IMPORTANT ENZYMES AND OTHER PROTEINS

243. INVESTIGATION OF ABNORMALLY EXPRESSED ENZYMES IN MALIGNANT BLOOD DISORDERS

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OBJECTIVE: The objective is to define the role of enzyme markers particularly lysosomal in the diagnosis, prognosis and follow-up of malignant blood disorders and to correlate results with cell surface receptor studies.

APPROACH: The plan of the research is: (a) to study not only beta-D-hexosaminidase and alpha-L-fucosidase in leukemic cells but also various other enzymes, particularly other lysosomal enzymes, and (b) to extend the investigation to other types of leukemia, including those found in adult life and also the range of lymphomas.

Where abnormal enzyme components are found, their properties will be fully investigated. This information should show how these enzymes differ from their native forms.

Initial studies will be carried out to assess the reliability of different methods in identification of specific enzyme markers. Ideally a simple yet reliable method that is suitable for multiple samples is required. The enzymes, including beta-D-hexosaminidase and alpha-L-fucosidase, will be separated into their component forms by isoelectric focusing, DEAE-cellulose chromatography and other suitable techniques for the determination of their profiles. One of these techniques would be the high resolution obtained by isoelectric focusing in flat beds of polyacrylamide gel, which offers a particularly useful analytical approach where subtle changes may be present in cancerous cells.

244. METABOLIC FATE OF MAMMARY CELL SURFACE GLYCOCONJUGATES

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Sialyltransferase has been found to be elevated in the sera of breast cancer patients. Specific cancer associated isoenzymes may be present which could be used as markers for breast cancer. Our initial studies using preparative flat bed isoelectric focusing did not indicate any novel isoenzymes of sialyltransferase in sera from breast cancer patients. Preliminary results have indicated two major serum sialytransferase enzymes with PI's of 4.9 and 5.3 in normals and cancer patients. However, in those studies a significant amount of enzyme activity was not recovered from the plates. Therefore, several new techniques will be attempted to increase recovery of enzyme activity while still maintaining good separation and fine resolution. These proposed studies include use of photoaffinity labeled substrates and new techniques in column isoelectric focusing. These analyses should provide definitive information on the usefullness of serum sialyltransferase isoenzyme assays in breast cancer.

Other studies will be performed which will assess the serum sialyltransferase inhibitory activity of several nucleoside-sugar antibiotics. The chemotherapeutic potential of these compounds and other N6-substituted adenosine analogs will also be evaluated in rodents with several transplantable mammary tumors. These studies should lead to the development of better therapeutic strategies in the diagnosis and treatment of breast cancer.

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245. PRODUCTION OF ALKALINE PHOSPHATASE ISOENZYMES BY HUMAN MALIGNANT CELLS IN CULTURE

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The objectives of this project are to examine the alkaline phosphatase isoenzymes produced by tumor cells in culture and compare them to normal tissue-specific isoenzymes of alkaline phosphatase. Differences in either the polypeptide or carbohydrate portions of these enzymes could be exploited in the development of more specific tumor markers, and could prove useful in comparing glycoprotein synthesis and processing in normal and malignant cells. Tumor cell alkaline phosphatases are being compared to their normal counterparts by differential inhibition with noncompetitive stereospecific inhibitors, heat and urea stability, immunoreactivity, chromatographic behavior, and electrophoretic migration under nondenaturing conditions. In addition, the isoenzymes can be radiolabeled in vitro with phosphorous-33, or in vivo with radioactive amino acids or sugars. Such radiolabeled products are being analyzed by SDS-polyacrylamide gel electrophoresis. Several tumor cell lines were found to produce isoenzymes similar in many respects to term placental

alkaline phosphatase. Peptide mapping studies showed that the polypeptide portions of these enzymes were very closely related. However, in many cases this tumor cell enzyme differed significantly from the placental form in catalytic properties, size, or degree of glycosylation. These data suggest that differences in the post-translational processing of the placental alkaline phosphatase polypeptide in tumor cells may result in altered catalytic or biochemical properties of this enzyme. The applicability of these differences to the development of tumor-specific alkaline phosphatase isoenzyme assays, as well as the molecular control mechanisms involved in the generation of these differences, are currently being investigated.

246. SERINE PROTEASES IN HOST-TUMOR INTERACTIONS

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Serine proteases that function as specific activators of plasminogen have a circumstantial association with neoplasia. This recommends them for study by those with an interest in the biology of malignant cells. Plasminogen activator synthesis by a variety of normal cell types is susceptible to modulation by physiological concentration of hormones or other biologically active compounds, a fact that has important implications for the study of abnormal control mechanisms in malignant cells.

The aim of the program is to verify 'in vivo' the presence and localization of serine enzymes in human and experimental tumors by autoradiographic techniques using tritiated diisopropylfluorophosphate (3H-DFP) as a marker of serine enzymes. Results in human Hodgkin's and non-Hodgkin's lymphomas showed high labeling of reticular cells and epithelioid venules of lymph nodes while lymphocytes showed low or negative labeling. Labeling was particularly heavy in lymphomas of a high degree of malignancy such as immunoblastic sarcoma and centroblastic lymphoma.

'In vivo' labeled enzymes are detected by fluorography after SDS gel electrophoresis and plasminogen dependent fibrinolysis is tested by the fibrin plate method. The molecular forms of plasminogen activator are detected by the zymographic technique after isoelectric focusing. Experiments on human breast cancers are in progress. The evaluation of the results will be possible on the basis of a wider selection of stages as well as histologic types.

247. PEPTIDE MARKERS OF CANCER DERIVED FROM TUMOR-ASSOCIATED PROTEASES

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Malignant tumors have been shown to contain elevated levels of proteases. The objective of this study is to develop markers of cancer based on protease degradation products that appear in human physiological fluids. Two approaches are being used. One approach assumes that a tumor-associated plasminogen activator would generate plasmin and that products of plasmin(ogen) autolysis would appear in physiological fluids of cancer patients. These peptides have either been chemically synthesized or isolated from purified plasminogen in our laboratory and radioimmunoassays (RIAs) are being established for them. Cancer patients' fluids will be assayed using these RIAs. A second approach consists of looking for low molecular weight peptides (less than or equivalent to 30,000 Mr) in cancer patients' fluids using the highly sensitive and selective techniques of high pressure liquid chromatography and countercurrent chromatography. The uniqueness of isolated peptide fragments to the cancerous state will be ascertained by examining fluids of normal and non-malignantly diseased subjects.

248. SERUM AMYLOID A

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During the past year the investigations carried out within this project led to results of some interest in two areas. Firstly, the serum amyloid A level was found to be a close monitor of cancer dissemination and of the response to therapy of patients with metastatic neoplasia. SAA levels above 400 ng/ml were found in 97% of 289 patients with solid tumors with distant metastases, in patients with acute and chronic myelocytic leukemia and patients with advanced lymphoma. They were lower than 400 ng/ml in 250 out of 270 patients with solid malignant tumors with localized and regional disease free of inflammatory processes. Among 20 patients from the latter group with SAA level higher than 400 ng/ml, 16 developed metastases within 214 days from the initial determination. The SAA level decreased in patients who responded to chemotherapy. A statistically significant correlation (p is less than 0.01) was established between the level of SAA and the size of measurable tumors. Secondly, it was established that among various subpopulations of peripheral human leukocytes the polymorphonuclear leukocytes or granulocytes are capable of synthesizing an AA antigenically related material designated granulocyte amyloid A (GAA). It was found that granulocytes, separated from 50 ml heparinized blood from 5 patients with high SAA levels, were able to incorporate 14C leucine (1 microcurie/ml McCoy medium free of leucine) in the de novo synthesized GAA detected in the solubilized granulocyte pellet and in their supernatant with the help of a double radioimmunoassay. The amount of intracellular 14C GAA was found to rise from 155 to 2200 cpm/4 x 10 to the 6th power cells over a period of 20 hours, then to decrease, while the extracellular 14C GAA increased proportionally with the percentage of dead leukocytes. Puromycin and cycloheximide significantly inhibited the synthesis of 14C GAA, vincristine and colchicine (antitubulin drugs) primarily blocked GAA release from the cells, while hydrocortisone increased its release and endotoxins in small doses increased its rate of synthesis. Preliminary data indicate that GAA is a protein of intermediate molecular weight (approximately 100,000 daltons). (Text Abridged.)

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249. PROTEIN SAA IN NEOPLASTIC DISEASE

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The objectives of this research proposal are to determine serum levels of protein SAA in neoplastic diseases, relate these data to diagnosis and prognosis, and define the relationship between this protein and the immune dysfunctions noted in cancer. Specifically studies are planned to determine if SAA is synthesized by neoplastic cells or is a factor synthesized as a reaction to a neoplasm. SAA is a serum alpha globulin known to be present in increased amounts in inflammatory diseases as well as in cancer. It has previously been shown to suppress the in vitro formation of antibody by CBA/J murine splenic lymphocytes.

Sera will be collected from patients with cancer of lung and gastrointestinal tract and SAA levels determined by radioimmunoassay. SAA levels will be correlated with histologic type of tumor, presence of metastases, response to therapy and recurrence of tumor. The relationship of SAA levels to abnormalities in lymphocyte function will be determined by measuring the PBL response to mitogens and the in vivo production of antibodies to tetanus toxoid in these patients.

The effect of SAA on the in vitro anti SRBC response of human peripheral blood lymphocytes (PBL) will be characterized using a system currently in use in this laboratory. PBL are cultured with SRBC antigen and the plaque forming cells enumerated by the Jerne assay four days later. Purified human SAA will be added to cultures of PBL in varying concentrations and the effect on response to SRBC measured by hemolytic plaque assay. The objective of these studies are: 1) to further characterize the suppression of immune function by SAA, 2) correlate this with the in vivo SAA level, and 3) determine whether neoplastic cells synthesize SAA.

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250. PROTEIN SAA IN NEOPLASTIC DISEASE

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The overall objective of this project is to define the relationship of serum amyloid A protein (SAA) to neoplastic disease. Data has been accumulated on an increased number of patients with carcinoma of the lung. These have shown invariably high SAA levels without obvious correlation with cell type. To date correlation of SAA level and mitogenic response of peripheral blood lymphocytes to phytohemmagglutinin has not been obvious.

Studies on the in vitro effects of SAA on response to T dependent antigen in the murine system of amyloidosis have shown that the response to sheep red blood cells can be partially restored with either normal T cells or normal macrophages. Complete restoration of mitogenic response after incubation with SAA can be restored by a combination of T cells plus macrophages. This suggests that SAA may cause immunosuppression by affecting T cell-macrophage interaction.

Continuing studies are planned to evaluate SAA levels and longevity with or without metastases in carcinoma of the lung.

251. PANCREATIC SECRETORY PROTEINS IN CANCER OF THE PANCREAS

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The aim of the proposed work is to analyze pancreatic secretions of patients suspected of having cancer of the pancreas or with a positive diagnosis of cancer of the pancreas and to compare them with secretory profiles obtained from healthy volunteers in the hope of detecting qualitative or quantitative abnormalities that might help in the early diagnosis of this disease. These studies will be accompanied by a similar investigation in hamsters during induction of pancreatic neoplasms with nitrosamines. Pancreatic tissue and fluid of groups of treated and untreated animals will be analyzed at regular intervals for changes in various parameters which in human patients have indicated the presence of pancreatic disease. Thus, ratios of trypsinogen variants, trypsin inhibitor, chymotrypsinogen and a number of lysosomal enzymes will be determined. Highly sensitive methods developed previously for investigation of human pancreatic fluid will be adapted for pancreatic tissue and fluid from hamsters. Data obtained from these assays will be correlated with concurrent histological studies in order to explore the possibility that changes in secretory proteins may precede recognizable histological abnormalities characteristic of tumor cells. Such changes or combination of changes might serve as sensitive markers for present or incipient pancreatic pathology.

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252. EXOCRINE PANCREATIC PROTEINS IN PANCREATIC CANCER

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Two-dimensional isoelectric focusing/sodium dodecyl sulfate gel electrophoresis will be used to characterize proteins contained in pancreatic cancer. Unique proteins will be identified using reductive methylation with H14CHO prior to separation on two-dimensional gels and analysis by fluorography. Proteins will be analyzed for attached carbohydrate of either the complex saccharide or core saccharide type by specific radioactive-labeling techniques. Immunofluorescent localization studies will be carried out to determine the site of origin of unique (glyco) proteins. If antigenic proteins are localized to pancreatic adenocarcinomatous tissue, a radioimmunoassay will be developed using cell hybridization and cloning techniques to provide the required antibodies.

The two-dimensional gel technique will also be used to analyze phenotypic changes among secretory proteins in two animal models of pancreatic adenoma and adenocarcinoma.

253. PANCREATIC DUCTAL VERSUS DUODENAL SECRETIONS

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The authors are attempting to find tumor markers in pancreatic secretions obtained from human beings at surgery and by ERCP, then to separate the proteins by isoelectric focusing and, finally, analyze the protein patterns using a densitometer which has been coupled with a small computer. The computer then averages and determines statistically the gravities of proteins in each peak, and compares normal secretions from those of patients with cancer and chronic pancreatitis.

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254. PROTEINS IN MULTIPLE MYELOMA AND RELATED BLOOD DISEASES

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The overall objectives of the research program entitled 'Proteins in Multiple Myeloma and Related Blood Diseases' will be to acquire further knowledge of antibody structure and diversity through correlative immunochemical, biochemical, physicochemical, genetic, and functional properties of the homogeneous immunoglobulins (Bence Jones proteins, myeloma proteins, and Waldenstrom macroglobulins) found in patients with multiple myeloma and related B cell malignancies. Studies of the monoclonal immunoglobulin components in these and other patients with neoplastic and inflammatory diseases are proposed to provide further information on the diagnostic, therapeutic, and prognostic importance and the functional significance of such proteins as biomarkers of cancer and of the humoral immune system.

The specific aims will be directed toward the establishment of concordance between immunochemical and structural features of human immunoglobulins, with emphasis on utilizing immunochemical techniques to provide a rapid and sensitive means for detecting and localizing structural differences in the amino-terminal or variant half (VL) and in the carboxyl-terminal or constant (CL) half of the light polypeptide chains of immunoglobulins; the characterization of physical, chemical, immunochemical, genetic, and other biologic properties of the V and C domains of light chains; the study of the formation and functional significance of the homogeneous units, subunits, and fragments of immunoglobulins found in the serum or urine of patients with B cell-related malignancies and of patients with other types of cancer of inflammatory diseases; and to ascertain further the therapeutic and prognostic usefulness of monoclonal immunoglobulins as biomarkers of cancer, especially to determine the frequency and significance of chemotherapeutically-associated immunoglobulin alterations as presaging the development of a second malignancy in a patient with cancer.

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255. ABNORMAL PROTEINS IN MULTIPLE MYELOMA

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This project is a continuation of a previous program designed to achieve the following ends: (a) to isloate and characterize the abnormal immunoglobulins appearing in the blood and urine of patients with multiple myeloma, macroglobulinemia, and related lymphomas; (b) to study the chemical, physical, and immunological properties of immunoglobulins IgM, IgA, IgG and IgD and also their relationships to each other and to Bence Jones proteins and normal immunoglobulins; (c) to do complete amino acid sequence analysis of Bence Jones proteins and the light chains of the immunoglobulins; (e) to undertake complete amino acid sequence analysis of the heavy chains of IgA, IgD, and IgG myeloma globulins and of pathological IgM human macroglobulins. The overall purpose is to ascertain the structural relationships among these proteins in relation to the problems of immunoglobulin biosynthesis, evolutionary development,genetic control, and antibody specificity and to develop information, reagents, and procedures of value for study of lymphomas and autoimmune diseases.

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256. THE ADENYLATE CYCLASE PROTEIN KINASE SYSTEM, STEROID RECEPTORS AND RETINOIC ACID-BINDING PROTEIN IN HUMAN MAMMARY TUMORS

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Recent experimental and clinical findings suggest that the presence of steroid hormone receptors in human mammary tumors is no longer sufficient to classify them as hormone-dependent, since a substantial number of hormone-independent tumors also contain significant amounts of estrogen receptors. Estrogen and progesterone receptors, at present, are still the most defined biological tissue markers with respect to their prognostic values. Nevertheless only 50% of the patients in whose tumors estrogen receptors can be found benefit from endocrine therapy.

Only limited data are available concerning the biological action of non-steroid hormones (i.e., prolactin) on neoplastic mammary growth. The generally accepted mechanism for these hormones is the binding to a tissue-specific receptor on the plasma-membrane followed by the activation of the adenylate cyclase/protein kinase system and the phosphorylative and functional modification of specific cellular proteins. Through the work of Cho-Chung an antagonistic action of the two hormonal systems in hormone-dependent tumor regression is suggested. In addition, recent findings give evidence that failure of hormone-induced tumor regression is due to the alteration of the regulatory subunits (cAMP-binding proteins) of the cAMP-dependent protein kinaes.

This research is designed to evaluate the detection of defective cAMP-binding proteins in human mammary tissue for prognostic purposes with respect to endocrine treatment as well as to evaluate steroid receptors, cAMP-dependent protein kinases (ratio type I/type II), cAMP-binding proteins (R1 and R2), and the retinoic acid-binding protein (CRABP) as biological tissue markers for the early detection of cancer in patients with dysplasia.

257. ALTERED CAMP-BINDING SITES IN OVARIAN-INDEPENDENT MAMMARY TUMORS

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Prediction of the responsiveness of human breast cancer to endocrine therapy is a major problem in the treatment of this disease. This research will use DMBA-induced rat mammary tumors to identify and characterize a potentially useful marker of endocrine-unresponsive disease. The presence of estrogen and progesterone receptors in breast cancer biopsy samples has been a useful prognostic indicator of endocrine-responsive disease, and recent preliminary evidence suggests that elevated cAMP-binding activity is associated with endocrine-unresponsive disease. The purpose of this research is to investigate thoroughly the reported cAMP-binding increase in DMBA-mammary tumors which do not regress following ovariectomy. These binding sites are most likely Type II, cAMP-dependent protein kinase. The present research will characterize the amount, identity, and chemical and catalytic properties associated with the elevated cAMP-binding activity in ovarian-independent DMBA-rat mammary tumors. If any consistent abnormalities exist, then this would provide a rationale for future studies to identify such abnormalities in human breast cancer.

258. VASOACTIVE INTESTINAL PEPTIDE, LEUKOCYTES, AND LEUKEMIA

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Vasoactive Intestinal Peptide (VIP) is a 28 amino acid peptide previously found in highest concentrations in central and peripheral nervous tissue. Although its physiologic role is not known, VIP is thought to be a neuromodulator of intestinal secretion. Using a highly specific radioimmunoassay (RIA) for VIP, we have demonstrated the presence of VIP cross reactive material (VIP-CRM) in a non-innervated tissue; namely, human polymorphonuclear leukocytes (PMNs). VIP-CRM was not measurable in lymphocytes, monocytes, platelets or erythrocytes suggesting this material may be a specific cell marker for PMNs. Further, our preliminary studies show that, although normal mononuclear cells (lymphocytes and monocytes) do not contain measurable VIP-CRM, mononuclear cells isolated from patients with either acute myelocytic leukemia (AML) or chronic myelocytic leukemia (CML) contained levels of VIP-CRM comparable to those levels observed in PMNs. These initial studies suggest VIP-CRM may be a biochemical cell marker specific for PMNs and their precursors. The purposes of the proposed research are: (1) to evaluate the clinical use of VIP-CRM as a biochemical marker in AML and CML; (2) to confirm the structural identity of VIP-CRM and; (3) to study the role of VIP or VIP-CRM in the function of PMNs and other peripheral blood cells.

259. VASOACTIVE INTESTINAL AND LUNG POLYPEPTIDES - TUMOR

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This proposal deals with the relationship of three newly identified polypeptides, vasoactive intestinal peptide (VIP) and two vasoactive lung peptides, to a number of malignant tumors. Using a sensitive and specific radioimmunoassay (RIA), VIP has been shown to occur normally in the gastrointestinal and nervous systems, and in elevated concentrations in these tumors: pancreatic (islet-cell) adenomas, pheochromocytoma, neuroblastoma, medullary thyroid carcinoma, and bronchogenic carcinoma. The clinical syndrome in common among these tumors was that of watery diarrhea (pancreatic cholera), accompanied in some instances by hyperglycemia, hypercalcemia, hypokalemia and episodic flushing. One vasoactive lung peptide, extractable from normal lung, is related to VIP, cross-reacting with it in the RIA. The other vasoactive lung peptide is spasmogenic and does not cross-react with VIP. Determination of the prevalence of secretion of these peptides in the presence of malignant disease may provide useful clues to the early diagnosis, better management and, possibly also, the pathogenesis of these lesions.

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260. PRESENCE OF A FOLATE BINDING PROTEIN IN NEOPLASTIC TISSUES

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The first objective of our project is to measure the folate binding capacity of the cytoplasm of neoplastic cells. The binding is due to a protein of 43,000 M.W. which seems to regulate the synthesis of DNA. The method used is a radioassay based on the use of radioactive folate as tracer, and a preparation of cytoplasm supernatant as the source of binder. A modified system has been developed for measuring the same protein in the serum. It has been recently found that the level of the binder in serum of patients with several tumors is significantly higher than normal and seems correlated with the invasion of the tumor (Europ. J. Cancer, 1980). In a longitudinal study initiated five years ago, the patients with chronic myeloid leukemia with a high level of intracellular binder, continue to survive (Intern. Congress of Haemat., Montreal 1980). This result supports the hypothesis that cells with an elevated amount of binder proliferate slowly. We are now preparing an antiserum against the folate binding protein (FBP) which might be of use as a marker of myeloid cells with a high level of FBP.

Another line of study concerns the mechanism of folate transport into the cell. It is well known that this transport is a saturable process, temperature dependent. We have developed a method for preparation of purified membrane from myeloid cells, using a sucrose discontinuous gradient. It will be now possible to see whether the folate transport is mediated by the presence of a carrier protein.

261. HUMAN ISOFERRITINS AND THEIR USE AS BIOLOGICAL MARKERS OF NEOPLASIA

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OBJECTIVE: To characterize human isoferritins from different organs and to evaluate their usefulness as biological markers of neoplasia.

APPROACH: Acidic isoferritins have been reported as possible biological markers of neoplasia; however, technical difficulties in the production of antibodies specific for acidic isoferritins have prevented the clinical application of this assay. The chemical purification and characterization of ferritin and of isoferritins from target organs such as heart, placenta and liver is the first step towards this goal. Antiserum to acidic isoferritins will be produced, followed by absorption with normal liver ferritin. Enzyme immunoassays will be developed for acidic isoferritins. A study of the correlation with diagnosis, stage and other biochemical and immunological markers will be performed. The origin of serum acidic isoferritins will be studied with human tumor cells in culture.

PROGRESS: A longitudinal study of serum ferritin was performed on a group of leukemic and breast cancer patients at various stages of the disease. Using an ELISA assay we found that serum ferritin was a valuable adjunct for the follow-up of breast cancer patients. There was a poor correlation between CEA and ferritin levels. Heart and placental type ferritins were isolated and characterized, and specific antisera were obtained. Ferritin synthesis by lymphoma cells in vitro was found to be phase dependent; also the synthesis of ferritins by these cells in vitro without iron stimulation suggests that the elevated serum levels of ferritin observed and lymphoma in leukemic patients are due to a de novo synthesis.

262. TRANSCOBALAMIN AND LEUKEMIA

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Two groups of cobalamin binding proteins are found in human plasma: a group of glycoproteins, R-binders, whose function is unknown and transcobalamin II responsible for the transport of cobalamins into the cells. Both groups of proteins are found partly saturated with cobalamin in plasma.

During chronic myelogenous leukemia (CML) the R-binder concentration increased considerably, while the transcobalamin II concentration is increased in acute lymphoblastic leukemia. In patients with other forms of leukemia and lymphoma the concentrations of these proteins are increased variably. The goals of the project are to characterize the clinical significance of saturated and unsaturated cobalamin binding proteins in plasma during the course of CML with special reference to the metamorphosis in comparison with acute leukemias to study the possible origin of the R-binders, and to describe the changes of the cobalamin metabolism during the progress of CML.

263. CHEMISTRY, STRUCTURE AND BIOSYNTHESIS OF MAMMALIAN EPIDERMAL KERATIN FILAMENTS

Steinert, P. M., Peck, G. L., Idler, W. W., Cantieri, J., Gullino, M. I., Yuspa, S., Goldman, R. D., Dale, B. A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Dermatology Branch, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The polypeptide chains which comprise the subunits of the keratin filaments of normal human, murine and bovine epidermis have been isolated and characterized. The subunits polymerize in vitro into native-type filaments. The repeating structural unit of the filaments consists of three chains aligned side-by-side with two discrete regions of super-coiled alpha-helix interspersed by regions of non-helix. Higher orders of filament ultrastructure are beeng investigated using physiochemical and electron microscopic techniques as well as sequence studies on individual subunits. The keratin polypeptide subunits of abnormal and malignant human epidermis are different from those of normal epidermis and work is in progress to define these differences. Normal mouse epidermal cells grown in cell culture synthesize the keratin filaments. The appearance of these proteins will be used as biochemical markers for studies of in vitro carcinogenesis. The 10nm filaments of a variety of cell-types have been shown to be structurally similar to, but immunologically different from keratin filaments. A histidine-rich basic protein isolated from rat epidermis and the slightly different protein of mouse epidermis specifically aggregate keratin filaments and other 10nm filaments in a manner suggestive of an interfilamentous matrix component.

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264. MEMBRANE PROTEINS OF HUMAN LEUKEMIAS AND LYMPHOMAS

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The objective of this study is to examine membrane protein patterns of human leukemias and lymphomas in SDS polyacrylamide electrophoresis gels in order to identify subsets of these malignancies and to compare these tumor protein patterns to those of proteins of normal subsets of lymphocytes in resting states and after mitogenic and alloantigenic activation. These latter experiments can reveal whether tumors represent expansions of normal lymphocyte subpopulations at some physiological state. (35S)methionine internal labeling of lymphoblasts and SDS slab gel electrophoresis and autoradiography of their isolated membranes has demonstrated more than 100 proteins with variable patterns among cell lines. Subset-specific proteins have been identified: p110 on T cells, p65 on CEM only, p85 and p29,34 (HLA-DR) on B lymphoblasts. Dramatic day-to-day changes in membrane proteins of normal T lymphocytes as a function of mitogenic activation have been found. These studies will be expanded in order to define band patterns which are specific to subsets of lymphoid tumors, to identify subset-specific molecules which can be isolated for the preparation of diagnostic antisera, to evaluate whether tumor cells are 'frozen' at some differentiation step or whether they experience protein band pattern changes in tissue culture or upon stimulation with mitogens or with MLR culture supernatant factors. Diagnosis of subsets of acute lymphoblastic leukemia, chronic lymphocytic

leukemia and non-Hodgkin's lymphomas will be emphasized by comparison of membrane protein 'fingerprint' patterns in electrophoresis gels with the patterns observed during the clinical course of each patient.

265. THE ROLE OF FIBRIN DEPOSITION IN CANCER

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This research project seeks an understanding of the mechanism by which blood coagulation is activated in patients with cancer and the importance of fibrin deposition in the biology of neoplasia. Methods will be developed and applied for the identification of circulating activators of blood coagulation in the plasma of cancer patients. Characterization of the enzymes and substances involved in the ultimate generation of fibrin will be performed using immunologic and biochemical methods. The potential role of monocyte tissue factor activation in the generation of intravascular fibrinopeptide A (FPA) in these patients will be explored. Lymphocyte-monocyte interactions necessary for tissue factor activation will be assessed in vitro. Co-culture experiments mixing normal donor and cancer mononuclear cells will be used to search for suppression and/or enhancement of tissue factor activation. Immune complexes will be investigated in autologous culture experiments as a putative activator of monocyte tissue factor. Fibrinopeptide A levels will be followed in patients with advanced cancer, resectable breast cancer and acute leukemia to determine the prognostic significance of FPA levels. The kinetics and anatomic source (e.g. intravascular vs. extravascular) of FPA will be determined in patients receiving chemotherapy and with infectious complications. The effects of heparin, warfarin and the antiaggregating agent RA233 on circulating FPA levels will be evaluated in hopes of providing additional information for the treatment of cancer.

266. SIGNIFICANCE OF GP48 IN DIAGNOSIS OF BREAST CANCER

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The objective of this proposal is to characterize and to quantitate those glycoproteins which are synthesized and released by human breast cancer surgical specimens. Special emphasis is given to gp48 and related glycoproteins, which appear to be released in greater abundance from malignant than benign specimens.

To achieve this, we propose to biosynthetically label glycoproteins with 3H- or 14C- carbohydrate precursors using either established cell lines or organ cultures of human breast cancer surgical specimens. The labelled glycoproteins are characterized by two-dimensional gel electrophoresis. Those components which are related to known serum glycoproteins are immunoprecipitated and their antigenic relationships are further investigated. Glycoproteins which appear antigenicly unique will be purified from surgical specimens, using the biosynthetically labelled glycoproteins as tracers. Their relationship to normal membrane components derived from breast epithelial cells will be investigated using human milk fat globular membranes. The purified glycoprotein will be used for the production of immune antisera or monoclonal antibodies. After appropriate adsorptions, the specific antisera will be used to establish a quantitative immunoassay using either a nonradioactive immunodiagnostic procedure with rate nephelometry or a radioimmunoassay with the 125I labelled glycoprotein.

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267. DNA-BINDING PROTEINS IN HUMAN SERUM

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Human sera contains a unique set of proteins with affinity for DNA. Initial characterization of these DNA-binding proteins has indicated their universal occurrence and alterations of these proteins have been found in malignant diseases. The primary foci of this research are the complete biochemical, immunological, and genetic study of the major species of DNA-binding proteins resident in normal human sera and the identification and characterization of the DNA-binding proteins with potential as tumor markers or indicators of other disease states. Current studies will emphasize the nature of the DNA-protein interactions specific to the serum DNA-binding proteins with emphasis on the localization of the actual DNA-binding domains; such studies are ultimately directed toward a correlation of the DNA-binding ability of these proteins with physiological functions. Secondly, there will be a continued evaluation of the diagnostic or prognostic potential of serum nucleic acid-binding proteins. Thirdly, studies will be continued to correlate nucleic acid-binding proteins of nuclear origin with their serum counterparts.

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268. RIBONUCLEASES OF HUMAN SERUM AND URINE

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The objective of this research project is to evaluate human RNases as useful biological markers of malignant disease with particular emphasis on carcinoma of the pancreas. The value of the RNases in this regard is presently the subject of intense controversy.

Using urine as the source, the initial approach to this problem was to purify and characterize those RNases present in normal humans. These RNases could then be employed in comparison studies with the enzymes present in the urine and sera of patients with malignant and appropriate non-malignant diseases. Methodology employed in these comparison studies includes (1) general biochemical parameters such as molecular weights, amino acid and carbohydrate composition, electrofocusing profiles, amino acid sequences, etc., and (2) the use of antibody directed against the normal RNases to look for possible antigenic differences between RNases from normal versus diseased individuals. A final, clinical approach to the overall problem will include the measurements of serum poly(C)-specific RNase activity present in various samples, with the specific objective of evaluating this particular RNase activity as a biological marker for pancreatic carcinoma. A poly(C) hydrolysis assay, i.e. the catalytic method, as well as a radioimmunoassay, which employs antibody directed against normal urinary poly(C)-specific RNase, will be used in this approach.

Presently, purification and extensive characterization of the only two detectable RNases in normal human urine has been achieved (Cranston et al. (1980) Biochim. Biophys. Acta, in press). A major finding was that a poly(C)-specific RNase, called RNase C, was shown to be a sialylated glycoprotein with marked amino acid sequence homology with all known pancreatic RNases. Preliminary evidence obtained with antibody directed against this enzyme supports the conclusion that the poly(C)-specific RNase present in the sera of all humans thus far tested is antigenically identical to the purified, urinary RNase C.

269. ABNORMAL METABOLISM OF CANCER PATIENTS

Rudman, D., Emory University, School of Medicine, Dept. of Medicine, *1364 Clifton Rd. N.E., Atlanta, Georgia*, 30322, United States of America

This project is concerned with the isolation and characterization of cancer-related proteins which are excreted in the urine by patients with advanced cancer. For each purified protein, a specific radioimmunoassay (RIA) is developed. The usefulness of each new RIA in the diagnosis of neoplastic disease is then studied.

270. BIOLOGIC MARKERS IN BREAST CANCER PATIENTS

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OBJECTIVES: Determine the concentrations of a selected group of constituent in breast tissue obtained at mastectomy and assay for these same substances in the serum of the patients obtained before their surgery and then sequentially on a scheduled basis over a period of time. Our aim is to find a constituent in cancerous breast tissue that is present in a concentration different from that in normal breast tissue and then to try to establish if changes in these concentrations in the breast tissue are reflected in the serum and whether the course of the disease is reflected in the serum pattern. We are now routinely obtaining in our laboratory breast tissue for analysis of estrogen receptor protein. It is our aim to determine in the cytosol and pellet of these tissues a variety of substances whose assays are in use in our laboratory. In addition, we can make available specimens for analysis in other laboratories.

271. EFFECTS OF CIGARETTE SMOKING ON THE PANCREASE & BRONCHIAL TREE (RELATION OF THE PROTEASE-ANTIPROTEASE INTERACTION TO CANCER)(ABBREV)

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This application concerns our continuing studies on the influence of cigarrete smoking on the pancreas. We have found an increased 'leakage' of the secretory pancreatic proteins into the general circulation in heavy smokers stimulated with secretin.

One of our aims is to elucidate the pathophysiologic significance of this finding. The functional and morphological effects of cigarette smoke on rat and golden hamster pancreas will will be studied.

A second goal is to develop a simple, safe test which will reliably demonstrate early cellular damage in the exocrine pancreas.

The distribution of the secretory pancreatic proteins will be established in pancreas, serum and other body fluids. Radioimmunoassays developed in this laboratory will be used for the majority of the quantitative and qualitative analyses of these secretory proteins in the various body fluids. These proteins will be localized in the tissues and intracellularly using immunohistologic methods.

The necessary antisera are being produced in our own laboratory, often after detailed analyses of the structure and function of the antigen. The interactions between the proteases from the pancreatic gland, as well as granulocyte, and their endogenous inhibitor are disturbed by cigarette smoke. Determination of to what extent and how requires further clarification, especially since it has been suggested that disturbances of the protease-antiprotease balance may contribute to the development of neoplasia.

Further, we want to elucidate the influence of cigarette smoke products on the protease-antiprotease balance in both the pancreas and mucosa of the respiratory tract, and to correlate these findings with the development of morphologic changes.

Previously we have shown that the leukocytes of myeloid leukemia frequently lack specific collagenolytic activity and we wish to explore this in greater detail. We also want to study the pathophysiologic significance of increased serum concentrations of neutral granulocyte proteases, especially in relation to chronic myeloid leukemia and its influence, if any, on the coagulation factors, i.e. factor XIII.

Finally, as a new point in our projects, we want to identify and characterize the major macromolecular component in seminal plasma in haalth and disease. This may form the basis for an improved diagnostic procedure for inflammatory and neoplastic diseases in the urogential tract of man.

272. ENDOGENOUS PEROXIDASE - MARKER PROTEIN IN ESTROGEN-DEPENDENT TUMORS (HUMAN, MICE)

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This is part of a broader project. A summary of this subproject is not available.

273. SERUM HIGH DENSITY LIPOPROTEIN-2 IN CANCER PATIENTS

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This is part of a broader project. A summary of this subproject is not available.

3. BIOCHEMISTRY OF PROGNOSTICALLY IMPORTANT HORMONE RECEPTOR PROTEINS

274. ESTROGEN RECEPTOR STATUS AND ASPECTS OF NATURAL AND CLINICAL HISTORY IN BREAST CANCER

Cocconi, P. C., Canaletti, R., Bozzetti, C., Boni, C., Ceci, G., De Lisi, V., Di Blasio, B., Guazzi, A., Lottici, R., Mori, P., Centro Medico Oncologico, *Via Gramsci 14, c/o Ospedali Riuniti, 43100 Parma, Italy*

The general objective of our research is to analyze the relationship between estrogen receptor (ER) status, response to endocrine treatment and aspects of natural and clinical history in breast cancer. Our approach consists of carrying out a series of studies on singular aspects of the problem and, by combining the results, general conclusions are possible.

The following studies are now in progress: 1) evaluation of response rate and duration of response comparing combination chemotherapy (CMF) and the same chemotherapy plus endocrine therapy (tamoxifen) in advanced breast cancer; ER status will be analyzed whenever possible; 2) evaluation of response rate, free interval, site of recurrence and survival in locally advanced breast cancer comparing treatment with combination chemotherapy (CMF) or the same chemotherapy plus endocrine therapy (tamoxifen) before and after radical local treatment (preferably radical mastectomy); ER status will be analyzed in all patients; 3) relationship between ER status in advanced breast cancer and time and site of metastases; 4) relationship between ER status and response to chemotherapy in advanced breast cancer; 5) retrospective analysis, in a series of patients with advanced breast cancer seen and followed continuously in our institution, of relationship between previous response or no response to whichever type of endocrine treatment and response rate to chemotherapy, duration of response, free interval, duration of advanced disease and total survival.

At present, progress in some of these studies suggests: 1) rate of response to combined chemical-endocrine treatment is higher than to chemical treatment; 2) breast cancers with metastases at time of diagnosis seem more frequently ER positive; 3) response rate to chemotherapy is similar in ER positive or negative patients but in the latter complete responses are more frequent; 4) in patients previously responding to whichever type of endocrine treatment compared to non-responding, rate of response to chemotherapy is similar, time of chemotherapy treatment and duration of responses tend to be longer, free interval but mostly duration of advanced disease and total survival are statistically longer.

275. SOLID TUMORS AND STEROID RECEPTORS

Conti, C., Concolino, G., Marocchi, A., Sciarra, F., Bracci, U., Di Silverio, F., Tenaglia, R., Picardi, C., Universita degli Studi di Roma, Istituto di Clinica Medica V, *Viale del Policlinico, 00100 Rome, Italy*

Steroid Receptors and Breast Disease: The aim of the project is the selection of hormone-dependent breast cancers in order to establish the appropriate endocrine- or chemotherapy, and the identification of mammary fibroadenoma in patients at 'high risk' of developing neoplastic diseases.

APPROACH: ER, PR and AR are measured both in the cytosol and nuclear fractions of malignant (primary tumor and nodes) and non-malignant tumors.

PROGRESS: So far 52 breast cancer patients have been studied and the follow-up as well as survival of patients undergoing endocrine- or chemotherapy evaluated.

Steroid receptors have also been studied in 10 mammary fibroadenomas.

276. A COMBINED DETERMINATION OF STEROID RECEPTORS, HORMONE RESPONSIVENESS AND ANDROGEN ACTIVITY IN WOMEN WITH MAMMARY CARCINOMA

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OBJECTIVE: To obtain more significant and valid information to aid in the selection of patients for hormone therapy (ablative endocrine surgery or exogenous hormones).

APPROACH: The absence of complete correlation between the presence of ER and response to endocrine therapy would be explained by the failure of the receptor, evident in total cell homogenate, to complete its mediation of the hormonal action because of both trans-location defect and inability to bind to the chromatin. For this reason we have developed a method which allows separate quantification of cytoplasmic and nuclear steroid receptors and we have collected preliminary cases for analyzing the possible correlation between the presence of these receptors, various characteristics of the tumor and the host (age, histological type, size, clinical stage) peripherally determined androgens and labelling index as measured by incorporation of tritiated thymidine. Possible correlation of various parameters all together in relation to clinical response will be sought with a view to improve the prediction rate.

PROGRESS: Data are being accumulated for subsequent correlation with clinical response to hormone therapy.

277. STEROID RECEPTORS AND INDUCIBLE PROTEINS IN BREAST TUMORS

Duffy, M. J., O'Connell, M., St. Vincents Hospital, Dept. of Nuclear Medicine, *Elm Park, Dublin, Ireland*

OBJECTIVE: The aim of this project is to determine if estradiol inducible proteins are better markers for hormone dependent breast cancer than the estradiol receptor alone.

APPROACH: Estradiol receptors and the estradiol inducible protein (peroxidase and progesterone) receptors are being measured in both normal animal tissues and breast tumors. The correlation between the presence of these parameters in human breast cancers and response to endocrine therapy is being investigated. It is hoped to determine if the presence of the inducible proteins adds to the predictive ability of the estradiol receptor.

278. STEROID HORMONE RECEPTOR CONTENT AND PREDICTIVE TEST FOR CHEMOTHERAPY OF BREAST CANCER

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OBJECTIVES: a) Information on biochemically prognostic factors relevant to selection of patients for adjuvant postoperative therapy regimens. b) Planning of individual hormonal and/ or cytotoxic chemotherapy in breast cancer.

APPROACH: Hormone receptor assay (17-beta estradiol (ER), progesterone (R 5020) (PgR)-receptor): Receptor determination is done by the dextran charcoal adsorption technique (according to EORCT, 1973). Definition of receptor content (fmol/mg cytosol protein): ER (PgR)-negative: less than 5 (less than 20), -poor: 5-10 (20-50), -rich: greater than 10 (greater than 50).

In vitro chemoresistance test: Native, mechanically isolated tumor cell suspensions were tested for a common primary chemoresistance, using a simple standardized in vitro test system without trypsinizing procedures. Therefore the effect of adriamycin (ADM; 10 to the 4th power - 10 to the minus first power mg/ml) as a non-cycle specific compound will be studied on the 3H-uridine (UdR) incorporation in trichloracid precipitable cell material. Definition of chemoresistance: reduction of UdR-incorporation by ADM (10 to the minus 2nd power mg/ml) less than 25% compared with control values.

PROGRESS: Tissue of primary untreated breast cancer of different histology and tumor stage without distinct metastases was investigated in 53 patients (13 pre-, 40 postmenopausal): ER (PgR)-negative - 36% (54%), ER (PgR)-poor - 11% (20%), and ER (PgR)-rich - 53% (26%). There were more receptor-rich cases in postmenopausal, as compared to premenopausal, women. Out of a total number of 19 ER-negative cases 8 (42%) were chemoresistant (PgR: 10/19 equals 53%) in comparison to 24/28 equals 86% of the ER-rich cases (PgR: 8/9).

Lack of ER- and/or PgR-receptors in primary breast cancer tissue is associated in vitro with increased response to cytotoxic drugs.

279. OESTROGEN RECEPTORS IN HUMAN BREAST CANCER

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OBJECTIVE: To investigate the methodology of oestrogen receptor assays in relation to the prediction of endocrine sensitivity of human breast cancer.

APPROACH: Breast tumours will be divided into 2 portions. Slices are cut from one portion and incubated in vitro with (3H) oestradiol-17 beta. Subsequently the cell nuclei are isolated and the extent of intra-nuclear translocation of hormone is assessed. The remaining portion of tumour is homogenized and the homogenate is centrifuged to prepare a common supernatant for three further assays: (a) saturation analysis for empty receptor sites, (b) exchange saturation analysis for total receptor sites and, (c) sucrose density gradient analysis to characterise the molecular form(s) of receptor present. In order to develop this methodology, rat mammary tumour models are being studied initially, but eventually where possible, the results of the various assays will be compared in relation to the clinical response of the patient to endocrine treatment.

PROGRESS: Past experience indicates that empty oestrogen receptor sites are detectable in about 70% of human breast cancers (Clin. Chim. Acta 64, 203, 1975) though the level detected is influenced by various factors, e.g., endocrine status (Euro. J. Cancer 13, 223, 1977), blood content (Lancet ii, 1251, 1976) and cellularity (Br. J. Cancer, in press 1977). We have confirmed that receptor-negative patients fail to respond to endocrine treatment but neither the presence of empty oestrogen sites nor the quantity of such empty sites in a breast cancer appear to correlate with response.

280. RECEPTOR AND HORMONE STATUS OF ESTROGENS IN ENDOMETRIAL CANCER AND HYPERPLASIA

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OBJECTIVE: To investigate the relationship between receptor status and hormone steroid metabolism in women affected with endometrial carcinoma and hyperplasia.

APPROACH: The determination of estradiol-soluble and nuclear receptors, the study of steroid excretion profiles by gas chromotography and mass spectrometry and the determination by radioimmunologic assays of the plasma rates of E2, Pg and T in female patients and in a control population selected according to age and menopausal state.

We regarded as having positive receptor status tumors with E2-soluble and nuclear receptor values higher than 0.24 fmol/microgram DNA.

PROGRESS: Preliminary results have detected minor anomalies in the excretion profiles of groups of patients with intact receptor function.

Our subsequent research is aimed at identifying parameters based on the relationship between the various classes of estrogens which might be clinically useful.

281. RECEPTOR STUDIES IN HUMAN BREAST CANCER

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We have demonstrated that estradiol receptor from two lines of rat mammary tumor MTW9 differ by chromatographic, kinetic and DNA binding properties. MTW9-MtT does not regress after ovariectomy, MTW9-P does regress. MTW9-MtT, the rat mammary tumor grown by coimplantation of the mammosomatotropic tumor MtTW10, shows ovariectomy-induced regression when MtT is resected. MTW9-MtT tumor cytosol receptor shows much less binding to DNA than does receptor from MTW9-P; after resection of MtT, DNA binding is similar. These data suggest that the differences between tumor receptors observed in our animal model may allow differentiation between tumors which have estradiol receptor and either regress or fail to regress after ovariectomy.

We propose to study human breast cancers which contain estradiol receptor by the techniques described which differentiate responsive from non-responsive tumors. Differences in receptor behavior would then be correlated with response to endocrine treatment. If such a study of estradiol receptors in mammary cancer would identify hormone-responsive tumors, current therapy would be improved.

282. PREMALIGNANT HUMAN MAMMARY EPITHELIAL HYPERPLASIAS

McCarty, K. S., Duke University, School of Medicine, Dept. of Pathology, *Box 3711, Durham, North Carolina*, 27710, United States of America

OBJECTIVES: Extend observations on rodent tumors to include a few carefully selected tumors according to the following criteria: histology, and ultrastructure supporting an epithelial origin; demonstrated presence of more than 15 fm of estradiol and/or progesterone receptor per milligram of protein as either cytoplasmic or nuclear form of the steroid receptor; positive identification of K-casein and alpha lactalbumin by immunofluorescence techniques. Statistically analyze data from this project and compare them with similar data on malignant tumors examined in the course of an ongoing study of pre- and postmenopausal breast cancer.

283. STEROID SULFATION & ESTROGEN-BINDING IN BREAST CANCER

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The specific aims are: To correlate the steroid sulfotransferase activity and estradiol binding proteins in the primary tumor at the time of mastectomy with pathological staging of the disease and the rate of recurrence; and to evaluate whether measurements of these biochemical parameters are useful for predicting response to endocrine ablation when metastasis occurs in these patients.

284. HORMONE RECEPTORS AND PEROXIDASE IN NORMAL ENDOMETRIAL TISSUE AND ENDOMETRIAL CANCER

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This is part of a broader project. A summary of this subproject is not available.

285. ESTROGEN-RECEPTOR ASSAY IN THE MANAGEMENT OF BREAST CANCER

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This is part of a broader project. A summary of this subproject is not available.

286. COMPARATIVE STUDY OF ENDOMETRIAL CANCER PATIENTS USING RECEPTORS AND EXCRETION PATTERNS OF STEROIDS AS PROGNOSTIC FACTORS

Castagnetta, L., Albano, V., D'Agostino, G., Polito, L., Universita degli Studi di Palermo, Inst. of Biochemistry, Laboratory of Hormone Biochemistry, *Via del Vespro, c/o Policlinico, 90127 Palermo, Italy*

OBJECTIVE: To attempt a case-control study of a group of patients suffering from endometrial carcinoma, with a particularly poor prognosis, selected according to certain biochemical markers, in order to evaluate steroid metabolism in relation to clinical response.

APPROACH: Selection from among endometrial carcinoma patients of a group with receptor status and steroid excretion profiles which indicate a reduced or absent hormone sensitivity; identification of a control population composed of patients with endometrial cancer which can be defined biochemically as hormone-sensitive, also selected according to menopausal state, tumor grading and staging.

We intent to carry out in the two groups: 1) a follow-up study of the frequency and sites of relapse, the disease-free interval and survival time; and 2) subsequent evaluation of the excretion profiles of the steroid hormones to assess their modifications in relation to clinical response of patients.

287. PROGESTERONE-SPECIFIC PROTEIN IN HUMAN ENDOMETRIAL SECRETIONS

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The ultimate aim is to determine if malignant endometria produce progesterone-specific proteins that can be detected by radioimmunoassay and provide a diagnostic test for malignant neoplasia or an indicator of the response of a neoplasm to hormonal therapy.

We plan to (a) isolate proteins secreted into the lumen of the uterus during the progestational phase of the menstrual cycle; (b) develop a test for the detection of these materials; (c) determine the influence of steroids on their synthesis; and (d) identify which cell types synthesize them. The secretory proteins isolated from uterine washings will be purified and used as antigens in the rabbit. Antibodies will be used as reagents in immunological assays on biological fluids and tissue sections. Organ and monolayer cell cultures will be used to establish the effect of steroids on the synthesis of these proteins. Once the proteins have been characterized and identified as being progesterone-dependent in normal tissues, washes of cancerous endometria will be examined.

288. CLINICAL EVALUATION OF THE HORMONAL RECEPTORS AND THE CEA TISSUE VALUES IN RECTAL CANCER

Mazzeo, F., Bucci, L., Del Rio, G., Universita degli Studi di Napoli, Fac. di Medicina e Chirurgia, Dept. of Semeiotica Chirurgica, *via Orazio 31, 80100 Naples, Italy*

OBJECTIVES: A comparison of grading, CEA tissue values and cancer hormonal receptors. 2) A comparative study of grading and histotypes with a double blind evaluation of the pre-operative biopsy and specimen by different pathologists. 3) A comparison of grading, hormonal receptors, CEA tissue values, and T.N.M. classification, local recurrences and survival.

289. PROLACTIN RECEPTOR CONCENTRATIONS IN BREAST CANCER

Malarkey, W. B., Ohio State University, Columbus Campus, School of Medicine, Dept. of Medicine, *370 W. 9th Ave., Columbus, Ohio*, 43212, United States of America

Prolactin markedly influences many aspects of normal breast physiology which are similar to events associated with growth. In the study of women with cancer of the breast it would therefore seem relevant to quantitate the concentration of prolactin receptors in the breast tumor tissue. In order to measure breast prolactin receptor levels, we plan to develop a radioimmunoassay for solubilized prolactin receptor. The influence of circulating hormonal levels, including estrogens (E1, E2, E3), progesterone, testosterone, cortisol, and prolactin, on prolactin receptor level in malignant breast tissue will then be investigated and correlated with the patient's clinical course.

290. SPECIFIC MARKERS OF NEOPLASTIC CELLS

Salvatore, G., Vecchio, G., Gurgo, C., Bridges, S., Consiglio Nazionale delle Ricerche, Centro di Endocrinologia ed Oncologia Sperimentale, *Naples, Italy*

OBJECTIVE: The objective of this project is to identify macromolecules specific for neoplastic cells.

APPROACH: Two approaches have been devised and two systems used. A) The first approach will investigate the significance of our finding of a receptor for TSH (thyroid stimulating

hormone) on the surface of transformed cells which are non-thyroid in origin. This approach is based on the discovery that TSH increases virus production by a rat fibroblast cell line transformed by and continuously producing Moloney murine sarcoma virus. This effect is probably due to the interaction of TSH with a cell surface receptor which, according to our evidence, is also shared by the glycoprotein interferon. We will investigate 1) if virus production is also stimulated by other glycoprotein hormones, 2) the relationship between the receptor(s) for TSH and interferon, i.e., whether they are identical or only partially related, and 3) if the TSH receptor is present on other cell lines transformed by RNA tumor viruses and on their normal counterparts, and its relative abundance on both types of cells.

Our previous work (C. Gurgo, M. Bondurant, and S. Bridges, Cancer Research 38, 2473, 1978) has shown that the RNA isolated from the thymus of leukemic mice contains sequences homologous to the c-DNA of Moloney murine leukemia virus (M-MuLV) which are absent in the RNA of the corresponding non-leukemic controls, thus supporting the hypothesis that RNA sequences specific for transformation are expressed in the neoplastic state. Our immediate goal is to isolate such 'transformation-specific' molecules, using thymic cells from lymphomatous AKR mice and their normal counterparts and a neoplastic thyroid cell line and its normal counterpart. Our approach is to isolate total mRNA from transformed cells (i.e., from thymic cells of leukemic mice or from neoplastic thyroid cells) and transcribe it in vitro with reverse transcriptase to obtain c-DNA. To eliminate base sequences common to mRNA of the corresponding normal controls, the c-DNA will be hybridized with the mRNA isolated from the thymus of non-leukemic AKR mice or normal thyroid cells, respectively. (Text Abridged.)

291. GLUCOCORTICOID RECEPTORS IN NORMAL DOGS AND DOGS WITH LYMPHOSARCOMA OR LEUKEMIA

Bottoms, G. D., Purdue University, School of Veterinary Science & Medicine, Dept. of Veterinary Physiology & Pharmacology, *Life Sciences Bldg., West Lafayette, Indiana,* 47907, United States of America

OBJECTIVE: Quantitate glucocorticoid receptors in lymphocytes from normal dogs and dogs suffering canine lymphosarcoma or leukemia. Because of the primary role of receptors in the initial interaction of glucocorticoids with cells, the presence or absence of receptors in most cases may be correlated with hormone responsiveness. Data collected will be used as a data-base to determine if one can predict the responsiveness of canine lymphosarcoma or leukemia to glucocorticois therapy. If some dogs are found to lack glucocorticois receptors, it is unlikely that glucocorticoid therapy would provide beneficial effects.

APPROACH: Lymphocytes will be isolated (J. Clin. Lab. Invest. 21: 97, 1968) from normal dogs and those suffering canine lymphosarcoma or leukemia. Assays for steroid binding protein (SBP) in lymphocytes will be conducted as described by Lippman et al. (J. Clin. Invest. 52: 1715-1725). Cytosol will be incubated with various concentrations of H-dexamethasone in the presence or absence of a 1000-fold excess of nonlabeled dixamethasone. Specifically bound steroid will be obtained from total counts minus nonspecific binding. The total SBP activity in all groups of dogs will be compared, and correlations between SBP activity and responsiveness of glucocorticoid therapy will be determined.

PROGRESS: Glucocorticoid receptors in lymphocytes isolated from normal dogs and dogs with lymphosarcoma or leukemia were quantitated in order to see if one can correlate the number of receptors in lymphocytes with responsiveness to glucocorticoids. Circulating lymphocytes were isolated from four normal dogs by centrifuging blood samples on a ficoll-Paque gradient. Lymphocytes were suspended in Eagle's Medium and incubated at 37 degrees C for 40 min. with varying amounts of (3H) dexamethasone either with or without a 100 fold excess of unlabeled dexamethasone. Following incubation the cells were washed and counted for receptor-bound radioactivity. Repeated assays have shown that lymphocytes in these dogs contain approximately 9250 + 1000 dexamethasone receptor sites/cells. One dog with hyperactive adrenal glands (treated with ACTH for 4-6 weeks) had 5471 + 905 sites per lymphocyte. The number of receptor sites returned to normal after ACTH was discontinued. (Text Abridged.)

III. STUDIES ON PROTEIN SYNTHESIS, MODIFICATION, AND TURNOVER RELATED TO CANCER BIOLOGY

A. STUDIES ON THE ROLE OF T-RNA IN PROTEIN SYNTHESIS

292. CHARACTERIZATION OF TUMOR-SPECIFIC TRNAS AND THEIR FUNCTION IN TUMOR CELLS

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OBJECTIVE: Attempts will be made to identify structure difference in nucleotide sequence level of tumor-specific tRNAs which appear specifically in tumor cells as detected by their change of chromatographic profile. Secondly, we will attempt to investigate the function of those tRNAs - whether they have a specific role in tumor-specific phenotypic expression.

APPROACH: Both tumor-specific tRNAs and control tRNAs are purified by succesive column chromatography and polyacrylamide gel electrophoresis. Total nucleotide sequence of those tRNAs is determined by post-labeling technique, and modified nucleosides are also analyzed. Effect of tumor-specific tRNAs on protein synthesis is also studied using wheat germ protein-synthesizing system with defined messengers.

PROGRESS: Isoaccepting tRNA species for tyrosine, histidine, asparagine and aspartic acid that appeared in rat ascites cell were found to be due to specific under-modification of Q nucleoside in tumor cells. The presence of such under-modified tRNA can easily be detected by incorporation of radioactive guanine into tRNA by E. coli tRNA-guanine transglycosylase. Tumor-specific tRNAPhe was also found to be due to under-modification of Y base in tumor cells. Structure of under-modified Y base has been determined.

293. AA-TRNAS IN PROTEIN SYNTHESIS AND EVOLUTIONARY CHANGES IN AA-TRNA AND SYNTHETASE REACTIONS

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Relative utilization of isoacceptor aa-tRNAs in protein synthesis have demonstrated variations in the incorporation of the corresponding amino acid into protein even though the isoacceptors have the same codon recognition properties. Two minor Ser-tRNAs which recognize specifically the nonsense codon UGA have been shown to be conserved in higher vertebrates. Patterns in codon recognition of isoacceptor aa-tRNAs from Physarum polycephalum have been determined and the aminoacylation of 19 aa-tRNAs fractionated from several species under homologous and heterologous conditions have been examined.

BIBLIOGRAPHIC REFERENCES: Hatfield, D., Matthews, C. R. and Rice, M.: AA-tRNA populations in mammalian cells. Part I. Chromatographic profiles and patterns of codon recognition. Biochim. Biophys. Acta 564: 414, 1979. Hatfield, D., Richer, L., Lyon, J. and Rice, M.: AA-tRNA populations in mammalian cells. Part II. Relative utilization of mammalian Lys-tRNA isoacceptors in protein synthesis. FEBS Letters, 1980, in press.

294. ISOLATION AND CHARACTERIZATION OF NOVEL FORMS OF NONSENSE SUPPRESSOR TRANSFER RNAS

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In order to eventually be able to correlate altered nucleotide sequences of mutationally altered tRNAs with altered func-

tions and molecular interactions this project proposes in general: the utilization of nonsense codons at position 211 of the alpha subunit of tryptophan synthetase (of Escherichia coli), where the behavior of (virtually) all amino acids is known, to obtain the conversion of specific tRNAs to novel forms of nonsense suppressors; genetic, physiological, and biochemical characterization of the mutants; isolation and purification of the altered tRNAs by column chromatography and polyacrylamide gel electrophoresis, and determination of the change in nucleotide sequence and/or nature and extent of base modification.

295. MISSENSE SUPPRESSION AND MUTATIONALLY ALTERED TRNAS

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Our program represents a systematic, mutational approach to transfer RNA structure-function relationships. It starts with selections for specific genetic alterations (missense suppressors) which have significance in vivo, moves on to the identification of the molecular lesion and aims at a greater understanding of precisely what aspects of the structure of certain tRNA are involved in their different functions and interacti ns. It calls for: (1) the utilization of missense codons, mainly at position 211 of the alpha subunit of tryptophan synthetase, whc e the behavior of (virtually) all amino acids is known, to obtair the conversion of specific tRNAs to new coding and amino acid accepting specificities; (2) genetic, physiological, and biocnemical characterization of the mutants; (3) isolation and purification of the altered tRNAs by appropriate techniques such as column chromatography, polyacrylamide gel electrophoresis, etc., and determination of the change in nucleotide sequence and/or nature and extent of base modification; (4) correlation of altered sequence with altered functions and other changes in tRNA structure and molecular interactions.

296. STRUCTURE-FUNCTION RELATIONS IN TRANSFER RNA

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Our research efforts in the next grant period will be devoted to determining the structural features of initiator tRNAs which allow them to be distinguished from non-initiator tRNAs with respect to recognition by formylating enzymes, protein synthesis initiation factors and ribosomes, and to determining the structural basis for the differences in biological activity of prokaryotic and eukaryotic initiator tRNAs in bacterial and mammalian protein synthesizing systems.

The experimental approach is to prepare hybrid tRNA molecules in which part of the structure is derived from one tRNAMet species and part is derived from another. The general research plan is (1) to purify initiator and non-initiator methionine tRNAs from E. coli, yeast, and rabbit liver and to isolate specific fragments of each tRNA by known chemical and enzymatic cleavage procedures, (2) to prepare hybrid molecules by annealing appropriate fragments from different tRNA structures and covalently linking them together with RNA ligase, and (3) to examine the biological activities of the hybrid molecules.

We will also investigate the structural basis for the specific interaction of prokaryotic and eukaryotic initiator tRNAs with E. coli methionyl-tRNA synthetase. Alterations in the anticodon bases will be introduced using RNA ligase and the effect of the structural changes on the ability of the enzyme to recognize the methione tRNAs will be studied.

In addition, we plan to study the interaction of E. coli initiator and non-initiator tRNAs with E. coli RNA polymerase. The specificity of the binding of various tRNAs will be examined and the nature of the binding site on the enzyme will be investigated by chemical crosslinking of the protein and nucleic acid. The properties and mechanism of the binding of fMet-tRNAfMet and the effect of DNA template, nucleoside triphosphates, and active transcription on the interaction will be examined by fluorescence and fast kinetic techniques.

297. STRUCTURE AND STRUCTURE-FUNCTION RELATIONSHIPS OF INITIATOR TRNAS AND OF MITOCHONDRIAL TRNAS

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Because of their unique role during the initiation of protein biosynthesis, initiator tRNAs from both prokaryotic and eukaryotic sources are required to interact specifically with a number of proteins. Consequently, initiator tRNAs possess several biochemical properties which are different from those of non-initiator tRNAs. One of the major objectives of this proposal is to understand the relationship between the structures and these unique biochemical properties of both prokaryotic and eukaryotic initiator tRNAs.

A direct approach for this involves the isolation and study of the properties of mutant tRNAs. Site directed mutations on cloned DNAs will be used to generate different classes of mutant tRNAs: (a) those derived from bacterial non-initiator tRNAs which now possess some of the structural features of eukaryotic initiator tRNAs and (b) those derived from eukaryotic, or bacterial initiator tRNAs which have now lost the structural feature unique to each of these classes of initiator tRNAs. The function and detailed properties of these and other mutant tRNAs will be studied both in vitro and when possible in vivo.

Our interest in initiator tRNAs has led us to work first on N. crassa mitochondrial initiator and then on other N. crassa mitochondrial tRNAs. We have found that mitochondrial tRNAs possess structural features not seen so far in any prokaryotic or eukaryotic cytoplasmic tRNAs. Futhermore, the codon reading patterns and in some cases even the genetic code may be somewhat different in mitochondria. We have proposed that mitochrondrial tRNAs which contain U in the Wobble position of the anticodon can read all four codons for a certain aminoacid, whereas those containing a modified U (U*) are restricted to reading codons ending in A or G. We shall examine the correctness and generality of this hypothesis by (1) completing sequence analysis of the remainder of the relevant mitochondrial tRNAs and (2) attempted expression of cloned N. crassa mitochondrial tRNA genes in E. coli with the hope of obtaining mitochondrial tRNAs which contain U* instead of U in the Wobble position of the anticodon, followed by purification and in vitro functional studies on the tRNAs thus obtained.

298. STUDY OF METHYLATIONS IN NEOPLASIA

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Transfer RNA is the most complex biomacromolecule in both structure and function. It can regulate at the level transcription or translation, as well as enzyme activity. tRNA requires appropriate modification for its effective participation in its many interactions. The methylating enzymes, the most frequent modifying agents, are hyperactive in every malignant tumor and in every tumor there are a few altered isoaccepting tRNA's. We intend to continue our studies of the systems which control methylation of tRNA in normal and neoplastic cells. Changes in activity of the tRNA methyltransferases, and in activity of a competing enzyme, glycine methyltransferase, will be followed during tumor induction by a variety of carcinogens. Other enzymes involved in regulation of methyl metabolism, such as S-adenosylmethionine synthetase and S-adenosylhomocysteine hydrolase will also be monitored. The effects of the perturbation of normal cell metabolism on metabolic pools and tRNA structure will also be investigated. The metabolism of methylated purines derived from the turnover of tRNA will be studied. Cancer patients invariably have elevated serum and urinary levels of these compounds. Two such compounds, 1-methylguanine and 7-methylguanine, cause changes in the growth properties of normal cells in culture, including some instances neoplastic transformation, by an as yet unknown mechanism. The mechanism of pathogenesis by these metabolites which stem from the turnover of tRNA will be studied at the molecular level.

299. THE STUDY OF PEPTIDYL TRANSFERASE RECOGNITION SITES

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The chemical synthesis of 2'(3')-O-aminoacyloligoribonucleotides and 2'(3')-O-(N-acylaminoacyl)oligoribonucleotides with nucleotide sequences identical to or resembling those of the 3' terminus of AA-tRNA will be carried out. These compounds will be used as models of the 3' terminus of AA-tRNA or peptidyl-tRNA in order to study their involvement in the various steps of protein biosynthesis on ribosomes and their interactions with elongation factors (EF-Tu, P and G). Modified tRNA containing 'fraudulent' nucleoside units on the 3' terminus will be synthesized by combination of chemical and enzymatic methods. These modified tRNAs will be used for study of various subreactions of protein biosynthesis.

BIBLIOGRAPHIC REFERENCES: B.R. Glick, S. Chladek, and M.C. Ganoza, Peptide Bond Formation Stimulated by Protein Synthesis Factor EF-P Depends on the Aminoacyl Moiety of the Acceptor, Eur. J. Biochem. 97, 23 (1979).

300. METABOLISM OF ANIMAL CELL RIBOSOMES

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The project aim is to determine at the molecular level the mechanism of an inhibition of protein synthesis initiation which is activated in human and other mammalian cells by conditions which decrease the charging of tRNA with amino acids. We have recently determined that this regulation is mediated at, or prior to, the synthesis of the 40S-Met-tRNAi initiation complex. Our immediate goal in the next year is to determine whether the mechanism of the regulation depends on phosphorylation of initation factor eIF-2. If this is the case we will seek to identify the kinase involved and determine how its activation is related to the state of charging of tRNA.

301. CHEMICAL STUDIES ON TUMOR NUCLEIC ACIDS

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Studies on the structure of macromolecules in cancer cells are essential for defining the biochemical lesion(s) distinguishing cancer cells from normal cells. This research proposal concerns 3 related topics: 1) A methodological investigation of postlabeling procedures for the sequence analysis of non-radioactive RNA; 2) sequence analysis of specific tRNAs; 3) studies on the effects of antineoplastic drugs (e.g., 5-azacytidine, 5-fluorocytidine) on tRNA and DNA as well as tRNA methyltransferases and DNA methyltransferases.

COMMENTS: Methods to be developed for the structural analysis of minute amounts of non-radioactive RNA will entail 3H-labeling and 32P-labeling, enzymatic and chemical digestions, chromatographic and electrophoretic separations. We wish to determine sequences of tRNAs that are rich in 5-methylcytidine because 5-azacytidine and 5-fluorocytidine were found in our laboratory to inhibit specifically the formation of this compound in nucleic acids. Preparation of nucleic acids deficient in a single constituent may facilitate one definition of biological role(s) of this constituent. In vivo studies are planned to investigate the effects of 5-azacytidine and 5-fluorocytidine on tRNA and DNA. In vitro studies will be aimed at elucidating the mechanisms of inhibition of RNA and DNA methylation by the nucleoside analogs. The sequences of tRNAs from tumors will be compared with their normal counterparts to define tumor-specific tRNA alterations.

302. ENZYME REGULATION

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Biologically hazardous by-products result from the production of electricity by nuclear reactors as well as by coal and petroleum. One of the hazards resulting from appearance of such by-products in organisms is mutation. This research will assess the mechanism of expression of mutations and determine how the mutant expression can be counteracted. The biochemical abnormalities associated with the vermilion and purple mutants of Drosophila are partially or fully restored to normal by the presence of a suppressor mutation su(s)/sup 2/ . The goal of this research is to determine how su(s)/sup 2/ accomplishes the suppresion of vermilion and purple. We have recently found that purple causes a decreased amount of sepiapterin synthase and that suppression is accompanied by a change in tyrosine transfer RNA. Vermilion is known to have greatly decreased tryptophan oxygenase activity. This activity is restored by su(s)/sup 2/ and also by dietary means. We shall assess how the activities of these enzymes are regulated and how transfer RNA levels are related to this regulation. This system is unique among higher organisms since it allows a detailed study of the mechanism by which an organism can correct the damage done by mutation. Damage and alteration of DNA causes mutations; a number of means of repairing the DNA are under investigation in other laboratories. This study is addressed to alternate means of correcting mutational damage. Additionally the toxic role of cadmium and zinc is being assessed on Drosophila viability and on the enzymes and tRNA described above.

BIBLIOGRAPHIC REFERENCES: Jacobson, K.B., A New Substrate for Formylkynurenine Formamidase: N',N/sup alpha/-Diformylkynurenine/sup 1,2/, Archives of Biochemistry and Biophysics 186(1), Feb 1978. Bell, J.B., K.B. Jacobson and L.R. Shugart, Multiple Isoacceptor Forms of Several Transfer Ribonucleic Acids in a Mutant Yeast Strain, Canadian Journal of Biochemistry 65 (1), 1978.

303. TRNA, PROTEIN-NUCLEIC ACID INTERACTIONS

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This is part of a broader project. A summary of this subproject is not available.

B. STUDIES ON REGULATION OF PROTEIN METABOLISM

304. SELECTIVE INHIBITION OF COLON TUMOR PROTEIN SYNTHESIS

Boffa, L. C., Allfrey, V. G., Vidali, G., Rockefeller University, Lab. of Cell Biology, *66th & York Ave., New York, New York*, 10021, United States of America

The aim of the proposed research is to analyze the mechanism by which cyanate selectively inhibits protein synthesis in tumor cells. Particular attention is paid to reactions involving the cytochrome P450 fraction and Na cyanate which generate products that suppress the uptake of amino acids by tumor cells in culture.

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305. CONTROL OF ALBUMIN SYNTHESIS IN LIVER AND HEPATOMAS

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The overall objective of this project is to identify the molecular mechanisms by which a characteristic liver gene expression, serum albumin production, may be altered in the neoplastic state. Albumin is the major product of protein synthesis in the normal adult liver, but in various hepatomas its expression is usually reduced or completely repressed. The rate-limiting components in the post-transcriptional regulation of the albumin gene will be examined in the normal adult rat liver and compared with the corresponding elements in specific hepatomas which produce abnormally low amounts of albumin. A complementary DNA (cDNA) to the purified albumin mRNA has been

prepared, and will be used as a specific nucleic acid hybridization probe to determine the quantitative distribution of cytoplasmic albumin mRNA. The identification of a possible nuclear precursor to the mRNA will be attempted, and the nuclear-cytoplasmic transport of albumin RNA sequences will be investigated. Total cDNAs prepared against total poly(A)-containing mRNAs will be employed to examine the sequence complexity relationships between hepatomas and the liver. The general physical and chemical properties of albumin mRNA will be studied.

Albumin gene expression will be correlated with that of its embryonic analog, alpha-fetoprotein. The production of alpha-fetoprotein declines dramatically at birth but is re-expressed in most hepatomas. The mRNA for this oncogenic antigen will be isolated by polysome immunoprecipitation, and a cDNA will be synthesized. The quantitative distribution of alpha-fetoprotein mRNA will be examined in the hepatomas, normal liver, and the developing embryonic liver. The albumin-alpha-fetoprotein relationship is one of the most important model systems currently available for the study of the expression of fetal antigens in cancer. This investigation should lead to a better understanding of the regulatory mechanisms involved in normal gene expression, and molecular alterations that can occur during this process in cancer.

BIBLIOGRAPHIC REFERENCES: Liao, W.S.L., Hamilton, R.W. and Taylor, J.M. (1980). Amino acid sequence homology between rat alpha-fetoprotein and albumin at the COOH-terminal regions. J. Biol. Chem. 255: 8046-8059. Liao, W.S.C., Conn, A.R. and Taylor, J.M. (1980) Changes in rat alpha-fetoprotein and albumin mRNA levels during fetal and neonatal development. J. Biol. Chem. 255: 10036-10039.

306. BIOSYNTHESIS OF A BRAIN-SPECIFIC S100 PROTEIN IN A CLONAL LINE OF RAT GLIAL CELLS

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OBJECTIVE: The relative synthesis of the brain-specific S100 protein increases in clonal rat glial cells (C6) when the cells progress from logarithmic to stationary growth in monolayer culture. The induction of S100 protein is paralled by an accumulation of its specific mRNA. Intercellular contact has been postulated to be the stimulus for increased synthesis of S100 protein. We have attempted to define by biochemical and genetic approaches the steps in the pathway involved in S100 protein induction; specifically extracellular signals trigger cell surface changes which cause intracellular mediators to trigger molecular mechanisms of protein synthesis.

APPROACH: the lectin succinylated concanavalin A(succinyl Con A) induces the synthesis of S100 protein in logarithmic cultures of C6 cells. Drugs which disrupt microtubules inhibit the synthesis of S100 protein in stationary cultures of C6 cells. Colchicine inhibits the accumulation of S100 protein mRNA. We propose to assess genetically the role of succinyl Con A, Cyclic AMP and microtubules in S100 protein induction. Induction of '100 protein will be studied in C6 cell mutant clones resistant to colcemid, concanavalin A and 8-BrcAMP and attempts will be made to link biochemically the genetic lesions to S100 protein synthesis.

PROGRESS: Mutant C6 cells resistant to growth inhibition by Con A have been isolated. Mutant cells bind less succinyl Con A than do parental cells and in contrast to parental cells do not round up or increase the synthesis of S100 protein upon treatment with succinyl Con A. However, the relative synthesis of S100 protein increases in the mutant cells as a function of growth in monolayer culture. Therefore, the inductions of S100 protein in C6 cells by growth and by succinyl Con A respectively may be mediated by different mechanisms.

BIBLIOGRAPHIC REFERENCES: Marks, A., Law, J. and Mahony, J.B. (1981) Synthesis of a brain-specific protein in a lectin-resistant mutant of rat glial cells. Miami Winter Symposium (abstract, in press).

307. THE ROLE OF PROTEIN PHOSPHORYLATION IN THE REGULATION OF DIFFERENTIATED FUNCTIONS OF CELLS IN CULTURE

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The proposed studies are designed to further our understanding of the role of protein phosphorylation in the regulation of certain differentiated functions of eukaryotic cells. They are based on the premise that information pertaining to the fundamental regulation of differentiated functions will be applicable to the study of pathological states. The plan is to study (a) the role of protein phosphorylation in the biochemical actions of insulin and related growth factors in cultured adipocyte-like cells and (b) the role of cyclic (AMP-mediated and Ca2 ion-calmodulin-mediated protein phosphorylation in regulating phagocytosis, motility and secretion in cultured macrophage-like cells. Our approach is two-pronged: (a) to define the relevant protein kinases and their substrates and (b) to examine function and regulation in cultured cells whose physiology and susceptibility to genetic manipulation make them especially suited to these kinds of investigations. The ultimate goal is to be able to use this information to augment, inhibit or otherwise regulate these biochemical processes so that physiological consequences can be pharmacologically controlled. This long range plan will of necessity require the integration of a number of approaches including (a) analysis of the physiological and biochemical pathways involved in the particular protein phosphorylation reactions: the protein kinases, phosphoprotein phosphatases, their activators, inhibitors and substrates and (b) the design of drugs that will specifically alter some step in the protein modification reactions. Our present program is concerned primarily with (a).

308. TRANSLATION CONTROL IN RETICULOCYTES AND LEUKEMIC CELLS

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Interferon induces a protein kinase that is activated by double-stranded RNA in susceptible cells to phosphorylate the smallest alpha subunit of eukaryotic peptide initiation factor 2 (eIF-2). A different protein kinase specific for eIF-2alpha is activated by heme deficiency in reticulocytes. Both kinases appear to phosphorylate the same sites on eIF-2alpha with concomitant inhibition of protein synthesis, thereby exerting translation control through a common mechanism. Potentially, the state of phosphorylation of eIF-2alpha can be regulated by either protein kinases or phosphatase activity. Components involved in the regulation of both these enzymes will be investigated. For the phosphatase, two heat-stable peptides that each activate the enzyme for different substrates will be studied. The activators will be further characterized with respect to physical properties, interaction with the phosphatase, and the mechanism by which the reticulocyte eIF-2alpha protein kinase is activated and controlled. This kinase may not only be activated by heme deficiency but also in the presence of heme by heat in the order of 40 to 45 degrees. Work will continue on the isolation and characterization of two components, HS and HL, that are involved in this heat-activated system.

BIBLIOGRAPHIC REFERENCES: Grankowski, N., Lehmusvirta D., Stearns, G., Kramer, G., and Hardesty, B. (1980) The isolation and partial characterization of two substrate-specific protein activators of the reticulocyte phosphoprotein phosphatase, J. Biol. Chem. 255, pp. 5755-5762. Kramer, G., and Hardesty, B. (1980) Regulation of Eukaryotic Protein Synthesis, in Cell Biology: A Comprehensive Treatise, (L. Goldstein, D. Prescott, eds.) Volume 4, pp. 69-105.

309. SERUM PROTEIN SYNTHESIS BY MOUSE HEPATOMA CELLS

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The overall objective of this research project is to study the regulation of serum protein synthesis in cultured mouse hepatoma cells (Hepa). The experimental situations to be investigated are designed to simulate physiologic changes believed to regulate serum protein synthesis in the liver. Additionally, studies of the effects of specific antibodies on antigen synthesis will be conducted. The objective of these latter experiments is to

understand the role of antibodies in the development of hepatocarcinoma.

The cell line (Hepa) to be used in these investigations was derived from the mouse hepatoma BW7756 (Jackson Laboratory). These cells secrete albumin, alpha-fetoprotein and transferrin into the culture medium. The rates at which the proteins are synthesized and secreted depend upon growth conditions.

The following regulatory phenomena will be studied in Hepa: 1) autoregulation of albumin synthesis, 2) effects of essential amino acids on albumin synthesis, 3) effects of cyclic AMP on serum protein synthesis, and 4) the effects of antibodies to specific proteins on the synthesis of their antigens. The experimental parameters to be evaluated are 1) cellular rate of synthesis (molecules/cell/minute), 2) polyribosome size, and 3) translation rate (polypeptides/minute). These measurements will allow the calculation of two additional protein synthetic parameters: 1) the rate of ribosome initiation on specific mRNAs and 2) the number of functional mRNA molecules per cell.

310. CONTROL OF GLIAL FIBRILLARY ACIDIC PROTEIN FORMATION IN HUMAN BRAIN TUMOR (GLIOMA) CELLS

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This proposal is designed to study glial fibrillary acidic protein (GFAP) in human glioma cells, to demonstrate factors that effect the formation of the protein, to relate GFAP formation to growth characteristics of glioma cells in culture, and to examine whether any of these characteristics relate to the clinical course or therapeutic response of the donor. GFAP is a specific marker for astrocytes and is believed to be associated with the 9 nm cytoplasmic filaments. The protein will be localized by the immunoperoxidase method and quantitative determinations done by rocket immunoelectrophoresis. Six factors, known to affect glioma cell growth or differentiation, will be studied for their putative effects on GFAP formation. The factors to be studied include polyamine metabolism, low serum concentration in culture media, dimethylsulfoxide, butyric acid, cyclic 3'5 AMP, and amethopterin.

311. STUDIES ON PROTEINS OF PLASMA CELL CANCERS

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The goal of these investigations is to understand the factors which control the assembly of antibodies and their reactions with hapten. The assembly process is viewed as comprising (a) folding of the indivdual chains during and/or after synthesis, which may include closure of intrachain disulfides; (b) noncovalent association of the folded chains into a tetrameric structure and (c) covalent assembly through oxidation of the reduced half-cystine residues that form the correct interchain disulfides. Our previous work has demonstrated that covalent assembly in vitro takes place within an already non-covalently assembled tetramer. The proposed research seeks to probe, in as much chemical detail as possible, the details of interaction between isolated Fv fragments, different pairs of light and heavy chains, and modified heavy chains with light chains and light chain domains. The importance of establishing conditions for stable heavy chain-light chain combinations cannot be overstressed. Within the plasma cell, in serum and in various in vitro states, the association of heavy and light chains, or even light chain fragments, assures correct disulfide bonds, enhances solubility and protects against degradation. It is proposed also to determine whether mouse hypervariable sequences (corresponding to given specificities, such as anti-DNP, anti-phosphocholine, anti-dextran, etc.) when inserted into human framework regions, generate stable H and L chain conformations, and retain binding specificities of the original mouse proteins. We plan also to study refolding rates from denatured states of separated CL, VL and intact light chain, utilizing stopped-flow CD and stopped-flow fluorescence. Finally, in view of recent advances in understanding the structure of immunoglobulins genes, we wish to examine peptide fragments coded for by the VL and JL exons, to see whether the variable region of light chains contains an indepedently folded subdomain. The work is important not only for an understanding of the molecular basis of immunoglobulin self-assembly but has significant biomedical potential. In view of the recent mouse hybridoma techniques, the ability to incorporate specificities against tumor specific antigens and against toxins into stable human antibodies would represent a significant biomedical advance.

BIBLIOGRAPHIC REFERENCES: S. Beychok, in Proceedings of a Symposium Cells of Immunoglobulin Synthesis, B. Pernis and H.J. Vogel, eds., Academic Press, pp. 69-88 (1979): Comparative Aspects of In vitro and Cellular Assembly of Immunoglobulins.

312. REGULATION OF NUCLEIC ACID AND PROTEIN SYNTHESIS - A BACKGROUND STUDY RELATED TO THE BIOLOGICAL EFFECTS OF RADIATION

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This proposed program is an effort to uncover regulators of protein synthesis in living systems. It is hoped that a deeper understanding of the mechanisms by which such regulation is accomplished in normal cells will lead to a better perception of the aberrations which lead to the deficient protein synthesis associated with radiation damage, and the excessive protein synthesis of neoplasia. A particular dinucleotide (AP4A) has been discovered which is associated with rapid cell growth, and an effort is being made to define its function. A triadecamer deoxynucleotide has also been synthesized which may inhibit oncogenic viral replication.

313. CONTROL OF PROTEIN SYNTHESIS BY DOUBLE-STRANDED RNA

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The biological significance of double-stranded RNA (dsRNA) is reflected in the increasing evidence which indicates that dsRNA exerts regulatory functions in a wide range of eukaryotic biological systems including normal and tumor tissues, cultured cells, and cell-free extracts. Some of these effects include (1) the induction of interferon, (2) a cytotoxic effect on cells, (3) the suppression of tumor growth and development, (4) an enhancement of the immune response, and (5) the potentiation of inhibitory effects in cell-free extracts. One of the most distinctive effects of dsRNA is its capacity to promote an antiviral state in tissues and cells similar to that achieved by interferon. Significantly, treatment of cells with interferon causes the induction of several dsRNA-regulated components, two of which are potent inhibitors of protein synthesis. These are (a) a cAMP-independent protein kinase which phosphorylates the alpha-subunit of eIF-2, the protein chain initiation factor which activates initiator methionyl-tRNA; and (b) a (2-5A)n synthetase which polymerizes ATP into short oligonucleotides linked by (2'-5')-phosphodiester bonds of the form (pppA(2')p(5')A (2')p(5')A); these oligomers serve as activators of endonuclease activity.

In addition to interferon-treated cells, the two dsRNA-regulated activities are found in normal and tumorigenic cells, and in reticulocyte lysates. The physiological roles of these functions are not clear. The induction of these two activities in interferon cells suggests that they are involved in the development of the antiviral state. The presence of the two activities in normal cells suggests that they are also involved in normal physiological processes.

The primary objective of the proposed study is to determine the mechanisms by which dsRNA regulates macromolecular mechanisms with emphasis on the control of translation. The specific research goals will focus on the (a) isolation and characterization of the two dsRNA-regulated activities; (b) examination of their mechanisms of activation and expression; and (c) their physiological functions in normal, tumorigenic and virus-infected cells.

BIBLIOGRAPHIC REFERENCES: Daniel Levin, Vivian Ernst, and Irving M. London. Effects of the catalytic subunit of cAMP-dependent protein kinase (Type II) from reticulocytes and bovine heart muscle on protein phosphorylation

and protein synthesis in reticulocyte lysates. Journal of Biological Chemistry (1979) 252, 7935-7941. V. Ernst and D. Levin. In situ phosphorylation of eIF-2 in reticulocyte lysates. Fed. Proc. 38:2913, 1979.

314. RNA AND PROTEIN METABOLISM IN CULTURED CELLS

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The synthesis, processing, and utilization of informational and regulatory ribonucleic acids and proteins are studied in normal, mutant, and growth arrested cultured mammalian cells. The knowledge and techniques developed in this basic effort provide a base for defining the effects of energy-related pollutants on genetic expression in cultured cells and thereby for defining the carcinogenic and teratogenic potential of such agents. The nucleoprotein nature of small, methylated RNAs was demonstrated, and particulate tRNA containing complexes were found. The distribution pattern among particulate forms of 19 aminoacyl tRNA synthetases was determined in extracts of normal and mutant mammalian cells. Alterations in informational RNA synthesis and processing were demonstrated to occur in cultured mammalian cells as a function of position in the cell cycle, in cells exposed to moderate doses of ionizing radiation, and in cells exposed to low levels of cadmium.

315. MECHANISMS OF CONTROL OF PROTEIN AND RNA SYNTHESIS IN NEOPLASTIC GROWTH

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OBJECTIVE: To detect metabolic changes in tumor cells.

APPROACH: Measurements of RNA synthesis, t-RNA levels, enzymes of polyamine synthesis and degradation in dividing cells (tumorous and non-tumorous).

316. PROTEIN SYNTHESIS AND CONTROL OF THE CELL CYCLE

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Previous results have suggested that the rate of cell division in cultured cells is determined by the rate of accumulation of proteins required in the G1 phase of the cell cycle, with the rate of protein synthesis maintaining its active control function when the culture growth rate is altered. This hypothesis will be tested in human diploid fibroblasts, normal and malignant human epithelial cells, and two rapidly growing cell lines. The hypothesis implies a reciprocal-normal distribution of G1 phase times in continuously proliferating populations; and it predicts that when the culture growth rate is altered by a change in extracellular conditions, the new rate of movement through the G1 phase and the new rate of protein synthesis will be correlated. The hypothesis will be tested by determining (1) if a reduction in the culture growth rate is accomplished by reversible withdrawal of cells from the cell cycle, as would be expected from reciprocal-normal kinetics, (2) whether the G1 times of siblings are correlated, (3) what quantitative relationship is maintained between the rate of protein synthesis and the rate of passage through the G1 phase, and (4) if the kinetics of growth stimulation are consistent with the hypothesis.

BIBLIOGRAPHIC REFERENCES: Castor, LaRoy N. A cell cycle model based on variations in the inherited rate of passage through the G1 phase. J. Cell Biol. 83: 13a, 1979. Abstract.

317. REGULATION OF PROTEIN SYNTHESIS

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Attention will be focused on the regulation of synthesis of immunoglobulin with emphasis on J-chain synthesis in plasmacytoma cells. J-chain is thought to be associated only with polymeric immunoglobulin and is therefore thought to play a role in polymer formation. Attempts will be made to determine whether synthesis of J-chain is coordinated with synthesis of other immunoglobulins and at what level the synthesis of J-chain is regulated. Variants of plasmacytoma tumor lines with defects at different steps in immunoglobulin production such as heavy or light chain synthesis, transport and polymerization, and variants which have lost the capacity to synthesize J-chain shall be used to determine whether the block in the synthesis is at the transcriptional or translational level. The primary sequences of J-chains in several cell variants will be compared to examine the possibility that the difference in the primary sequence of J-chain may explain the variations in the polymerization, secretion and synthesis of immunoglobulin. Past-transcriptional modification of mRNA for J-chains as well as other immunoglobulin chains will be investigated.

318. REGULATION OF ALBUMIN SYNTHESIS BY AMINO ACIDS

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The in vivo synthesis of serum albumin by liver has been shown to be specifically controlled by plasma levels of essential amino acids especially tryptophan. The apparent relative stability of the albumin mRNA would suggest that these amino acid effects occur at the translational level. The overall aim of this research project is to analyze the effects of essential amino acid limitation on albumin synthesis, and to investigate the possibility of exogenous albumin as a source of essential amino acids. The focus of these studies will be a highly differentiated mouse hepatoma cell line, Hepa, which continues to secrete the serum proteins, albumin, alpha-fetoprotein, transferrin and complement C3b inactivator. The initial phase of the project will involve the systematic manipulation of culture media concentrations of tryptophan, histidine, isoleucine, leucine and phenylalanine. Absolute rates (molecules/cells/min) of albumin secretion will be measured by radio-immunoassay. The specificity of amino acid effects on albumin synthesis will be assessed by comparison with other serum proteins and total cellular protein. Kinetic analysis of albumin and total protein synthesis will include measurements of 1) relative and absolute (albumin) rates of synthesis, 2) ribosome transit times and 3) number-average polyribosome sizes. From these data mRNA translational efficiencies (initiation rates) and relative and absolute (albumin) cellular contents of functional mRNAs will be calculated. The data will be interpreted in terms of general translational control models.

319. ENZYME SYNTHESIS AND DEGRADATION IN CELLULAR REGULATION IN ANIMAL TISSUES

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Our studies are directed at the mechanism(s) for regulation of specific protein synthesis in higher eukaryote systems. The techniques we employ involve isolation and characterization of specific mRNAs and synthesis of complementary DNA which is used to obtain recombinant DNA molecules useful in the understanding of biochemical regulatory mechanisms.

We plan to use these techniques to understand the mechanism whereby cultured mouse and hamster cells, when grown in progressively increasing concentrations of the folate analog, methotrexate, develop high resistance as a result of amplification of the gene for the enzyme, dihydrofolate reductase. We have found that the genes can be stably or unstably amplified in different cell lines, and that this difference is a result of the genes being integrated into chromosomes or present as small, chromosomal elements called 'double minute chromosomes.' We plan to study the mechanism of the duplication, as well as the mechanism for the production of double minute chromosomes and their potential reintegration into chromosomes.

320. THE CONTROL OF MAMMALIAN PROTEIN SYNTHESIS AT A MOLECULAR LEVEL

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Plasma high density lipoprotein (HDL) has been shown to be the main source of cholesterol used by the rat adrenal cortical cell for the synthesis of steroid hormones. HDL is bound at a high affinity receptor site on the adrenal cortical cell membrane. HDL binding and uptake is stimulated by ACTH. HDL is transported from the cell surface to the lysosomes where the protein is degraded and cholesterol released. Cholesterol is then

transported to the mitochondria where it is converted by the cytochrome P-450 cholesterol side chain cleavage system to pregnenolone, the first steroid. Pregnenolone by multiple enzyme systems is converted to corticosteroids. It was observed by us that HDL, when incubated alone with adrenal cortical cells, induces a two-fold increase in corticosterone production. In the presence of both 3'5'-cyclic adenosine monophosphate (cAMP) and HDL, corticosterone production was stimulated 100 fold. Both in the presence and absence of cAMP, large amounts of cholesterol derived from HDL are taken up by the adrenal cell.

Apparently in the cell which is not stimulated by cAMP, cholesterol though taken up by the cell, is either not transported into the mitochondria, or if it is, is not bound to the cholesterol side chain cleavage system, and therefore not converted to steroids. It will be our next task to study the translocation of cholesterol from the cell membrane through the lysosomes to various other intracellular fractions. The intracellular fractions of the adrenal cells will be homogenized and separated centrifugally using the appropriate marker enzymes to determine the purity of these fractions. They will then be analysed for free and esterified cholesterol. This work will take about two years.

321. CHEMOTHERAPY WITH INHIBITORS OF PROTEIN SYNTHESIS

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The rational design of drugs to be used for chemotherapy is contingent upon (a) choosing a system which, if controlled, will have dramatic effects upon cellular function, and (b) achieving selective toxicity. The long-term objective of this proposal is to develop rational methods for control of cellular function which will demonstrate tissue and species selectivity and thus be of use in treatment of disease. Specifically, the systems which will be investigated were chosen because of their central importance in protein biosynthesis and regulation of growth control: selective modulation of these systems would be of great value to chemotherapy in general and cancer chemotherapy in particular.

We shall study a number of mammalian aminoacyl-tRNA synthetases. Our objectives are (1) to develop potent and specific inhibitors of these enzymes, (2) to demonstrate the efficacy and specificity of these enzymes in vivo, (3) to ascertain if these inhibitors induce central regulatory mechanisms of growth control, and (4) to ascertain whether they have a differential effect on normal and neoplastic cells. A hypothesis is forwarded that arrest of normal mammalian cells in the G1 interval of the cell cycle is accompanied by, and perhaps triggered by, increases in intracellular uncharged/charged tRNA ratios. Experiments are proposed which will support or negate this hypothesis, and we will attempt to induce this regulatory response with the specific inhibitors of the aminoacyl-tRNA synthetases we shall design.

Further, we are investigating the role of diadenosine tetraphosphate in cell growth and its possible role as mediator in the pleiotypic response; we are also investigating the biochemical mechanism of formation and destruction of this molecule.

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322. STUDIES ON LECTINS AND OTHER SUBSTANCES INHIBITING PROTEIN SYNTHESIS

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Aim of the project is the study of toxic and non-toxic lectins and other plant proteins which inhibit protein synthesis by inactivating ribosomes. Ultimate objective is the preparation of conjugates of these proteins with suitable carriers (antibodies, hormones) capable of killing tumor or other cells in a selective manner.

The main results obtained are: 1. The purification and characterization of modeccin, a potent toxin from Adenia digitata, the separation of its subunits and the identification of a second form of the toxin. 2. The purification and characterization of gelonin, a non-toxic inhibitor of protein synthesis from Gelonium multiflorum. Gelonin is not toxic to whole cells, since it does not bind to them, hence being unable to enter the cytoplasm. Gelonin was conjugated to concanavalin A through an artificial disulphide bond, and the resulting complex was toxic to HeLa cells. (This research was performed in cooperation with the Norsk Hydro's Institute of Cancer Research, Oslo). 3. Two new mitogenic lectins were purified from Vicia cracca and from Hura crepitans (Research performed in cooperation with the Department of Biochemistry, Faculty of Pharmacy, University of Bologna).

323. STUDIES ON PROTEIN SYNTHESIS REGULATION IN ACTIVELY PROLIFERATING TUMOR CELLS

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OBJECTIVE: mRNA stability and EF-2-ribosome association, in normal and neoplastic cells: possible role in the translational control of protein synthesis. Isolation of mRNAs which codify for specific proteins.

APPROACH: a) The mRNA stability is studied as function of the length of the PolyA tails. It is possible to postulate that different length of PolyA tails is responsible for a different stability of mRNa in normal and in tumor cells. This can be considered a possible mechanism of translational control in the neoplastic, actively proliferating cells. Techniques employed: total mRNA is isolated by cold phenol extraction, the purification of the PolyA containing species is made by Oligo-dT chromatography, mRNA is analyzed by sucrose gradients in SDS, PolyA-mRNA is digested by T1 and pancreatic nucleases and PolyA tail length is determined by polyacrilamide gel electrophoresis. b) The association of EF-2 (elongation factor 2) with different ribosomal populations is studied in liver and reticulocyte cells (as control system) and in the ascitic tumor cells of Klein (3-methylcholanthrene induced tumor) in two different functional conditions: 'Fed condition' and 'Starved condition'. The ribosomal populations - monomers, subunits, polysomes - are isolated and purified by zonal sucrose gradients. The EF-2 associated with the different ribosomal fractions is determined by the method of ADP-ribosylation by diphteria toxin. c) Isolation of mRNAs which codify for specific protein in tumor cells was undertaken in order to identify a gene product which could be responsible for the neoplastic transformation. Specific mRNAs are isolated by immunoprecipitation of the different fractions of ribosomes and the protein product is studied in a cell-free system directed by the natural mRNA purified from the immunoprecipitate.

324. REGULATION OF MAMMALIAN PROTEIN BIOSYNTHESIS

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OBJECTIVE: The aim of the project is to investigate several aspects of the regulation of protein synthesis in eukaryotic cells. It is proposed to analyze in depth the mechanisms which are involved in control of translation at the cytoplasmic level in normal animal tissues and tumor cells grown in tissue culture. The modes of action of agents which affect cellular growth and division, including interferon and nutritional factors, will be studied.

APPROACH: The work will involve the preparation of rabbit reticulocytes and the growth of mammalian tumor cells in tissue culture. Cell-free extracts will also be prepared from these materials. Such systems will be used to study the mechanisms of action of interferon as both an anti-viral and an anti-cellular (anti-tumor) agent, and also the regulation of polypeptide chain initiation by variations in nutrient supply. The mode of action of haem in controlling protein synthesis in reticulocytes will also be investigated further.

Two complementary approaches will be used, as appropriate. In the first, attempts will be made to correlate the behavior

of the intact cells with properties of the crude extracts derived from them. In the second, individual components of the protein synthesizing machinery (such as initiation factors and mRNA) will be purified and used to reconstitute in vitro systems for assays of partial reactions of protein synthesis.

Other aspects of the control of gene expression and cellular growth will also be studied in attempts to define the detailed mechanisms of action of interferon and of essential nutrient supply; DNA synthesis, RNA synthesis and the transmembrane transport of the precursors for macromolecular synthesis will be investigated in their appropriate contexts.

PROGRESS: The results which constitute the basis of this project show that protein synthesis in mammalian cells is regulated at the translational level primarily at the stage of polypeptide chain initiation. Control of cellular growth rates by variations in nutrient supply (e.g. essential amino acids), and of protein synthesis in reticulocytes by haem, both involve modulation of formation of (40S ribosomal subunit, Methionyl-tRNA) initiation complexes. (Text Abridged.)

325. REGULATION OF SPECIFIC PROTEIN BIOSYNTHESIS IN TUMOR AND NORMAL TISSUE

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A rat calcitonin-producing tumor line (MTC) will be used as a model system. This model system provides a number of advantages for study of certain aspects of regulation of protein synthesis. First, the mechanism by which calcitonin and other candidate hormones contained in a common precursor are cleaved and secreted represents an excellent model system for understanding the biosynthesis of small polypeptide hormones. The role of the Gly.Lys.Lys.Arg sequence in the N terminal amidation of the proline residue can be directly studied using a radiolabelled cell-free translation product. These tumors synthesize a series of neuroendocrine-active hormones, and the relationship of their biosynthesis can be initially defined. Finally, every instance studied of conversion from a high to a low production state with respect to calcitonin appears to involve alterations in processing of calcitonin mRNA transcripts rather than alterations in calcitonin gene transcription. This unexpected finding has interesting implications in regulation of eukaryotic gene expression. Elucidating whether this reflects an alteration in primary gene structure, or altered regulation of the mRNA processing pathway will be a major goal of this proposal. Finally, establishing permanent cell lines derived from these tumors will permit definition of the mechanisms by which hormones, calcium, and possibly cyclic nucleotides, modulate calcitonin gene expression.

326. A CELLULAR MECHANISM FOR CONTROL OF PROTEIN SYNTHESIS

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Experiments will be carried out to provide a detailed description of one of the cellular mechanisms of regulating the level of protein synthesis. Cellular levels of the enzymes and enzyme activators involved in this regulatory system will be compared for normal and tumor cells grown under sub-optimal conditions, such as crowding or starvation. The comparison of the responses of normal and tumor cells to these conditions should give us some insight into the reasons for the unrestrained growth associated with cancer cells, as well as knowledge which can be used in the design of anti-tumor drugs. This regulatory system involves the production of an oligonucleotide pppA2'p5'A2'p5'A, which activates an endonuclease that degrades messenger RNA and thereby inhibits protein synthesis. Since interferon is known to stimulate this system these experiments should also help us to understand its anti-tumor activity.

327. CONTROL OF SYNTHESIS OF A TRANSFORMATION-DEPENDENT SECRETED GLYCOPROTEIN

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Cultured mouse fibroblasts which are transformed by RNA viruses, a DNA virus or a chemical agent, all secrete a 35,000 Mr-glycoprotein (major excreted protein, MEP) in large amounts. Non-transformed murine fibroblasts secrete this protein in much lower amounts. These fibroblasts and other murine cells can be stimulated to release MEP by treatment with tumor promoters. We have purified this protein and prepared antisera against it. The protein, of unknown biologic function, undergoes extensive modification prior to secretion. We are studying this system as a model of regulation of protein synthesis, processing and secretion as it is affected by transformation and agents which mimic the transformed state, such as tumor promoters.

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328. GENETICS AND REGULATION OF MAMMALIAN RIBOSOMAL COMPONENTS

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Over the past few years we have been able to measure accurately the synthesis of individual ribosomal proteins. From a number of experiments in our lab and others', one concludes that there is a remarkable coordination in the synthesis of ribosomal RNA and ribosomal protein. This coordination occurs at three levels: coordination leading to equimolar synthesis of seventy ribosomal proteins; coordination leading to equimolar synthesis of ribosomal proteins and ribosomal RNA; coordination leading to parallel changes in the synthesis of ribosomal proteins and RNA in response to changes in environment. The experiments described in this proposal are designed to probe the basis for this coordination.

We have fused mouse 3T3 cells with Chinese hamster cells carrying the emetine resistance gene, and have demonstrated the resulting hybrid cells contain hybrid ribosomes. Although individual clones of hybrid cells have different ratios of mouse to hamster RNA, the hybrids synthesize, overall, equal amounts of RNA and proteins. We will ask whether the basis for the balance is at the level of the synthesis or the utilization of mRNA for ribosomal proteins, by analysis of the products of cell-free translation RNA from various hybrid lines. In particular we will compare two hybrid lines, in one of which the ratio of two hamster proteins is 1:1 and in the other is 2:1. We have designed a genetic approach to determine if there is a species specific master gene which controls the synthesis of all ribosomal proteins. One of the parental cells is transformed, while the other (3T3) retains a substantial degree of growth control. We will ask whether remnants of this growth control exist in the hybrid in terms of a relative inhibition of synthesis of mouse RNA or proteins in cells deprived of serum.

We propose an exploratory effort to isolate the gene for drug resistance by transformation. Finally, we propose to ask whether the gene for a yeast ribosomal protein, analogous to protein S6 of mammalian cells, can function in mouse LTK cells.

329. SKELETAL MUSCLE PROTEIN TURNOVER IN MALIGNANT CACHEXIA - AN INTEGRATED STUDY IN EXPERIMENTAL ANIMALS AND MAN

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The object of the present study is to determine the synthesis and breakdown rates for skeletal muscle protein in patients with malignant cachexia and to study the mechanisms involved in animals with tumor-induced wasting. A particular feature of proposed work is the measurement of muscle protein synthesis in patients in vivo by the use of stable isotope non-radioactive amino acids in a method recently developed by us. In the Department of Human Metabolism at University College Hospital we are uniquely placed to carry out integrated collaborative

studies in patients (with Dr. R. Souhami and Dr. A. Goldstone of the new Oncology Unit at UCH). The animal studies on tumor-induced wasting in mice are to be carried out in collaboration with Professor Munro Neville of the Ludwig Institute for Cancer Research, Sutton, Surrey. The personnel, expertise and analytical procedures are currently available in regular use. The study is aimed to provide a rational basis for nutritional management of cancer patients with a view to minimizing muscle wasting.

330. REGULATION OF CELL PROTEIN TURNOVER IN NORMAL TISSUES AND TUMORS

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OBJECTIVE: Normal cells are able to control their protein turnover rate with respect to the growth phase: protein degradation appears to be restricted in growing cells, definitely faster in quiescent cells. This control mechanism is impaired, to different degrees, in neoplastic cells. The role played by lysosomal proteinases in the regulation of the rate of cell protein catabolism in normal and tumor cells with respect to growth will be investigated, the aim being to find out whether cell growth can be controlled, pharmacologically for instance, through modulation of cell protein catabolism.

APPROACH: The working hypothesis is that the rate of cell protein catabolism is directly related to the intracellular activity (availability) of lysosomal proteinases and inversely related to growth. These relations are being investigated in different model systems, including quiescent and growing normal cells (rat liver, cultured cells) and neoplastic cells (induced and transplantable hepatomas, transformed and neoplastic cells in culture). The turnover of cathepsin D and possibly cathepsins B and L will also be measured in order to understand how their intracellular level is regulated.

PROGRESS: The activity of some lysosomal proteinases in the rat liver has been measured in different situations of tissue growth: compensatory growth after partial hepatectomy, developmental growth, synchronization by casein in week-old animals, hyperplasia in Yoshida ascites hepatoma-bearing rats. These activities have also been measured on cell subpopulations dissociated from the liver tissue and fractionated by isopyknic centrifugation or by sedimentation at gravity one. The results are consistent with the view that the activity of the proteinases is inversely related to growth and directly related to the rate of cell protein catabolism. These observations are being expanded to other cell systems, particularly tumor and transformed cells, in order to elucidate the contribution of reduced cell protein degradation to neoplastic growth and the role played by lysosomal proteinases in cell growth control.

331. A STUDY OF PROTEIN TURNOVER IN MALIGNANT DISEASE

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OBJECTIVES: a) To assess the reproducibility of measurements of protein turnover as made by the N15 glycine/urinary ammonia method. b) To assess the application of this method to clinical studies in malignant disease.

APPROACH: a) Factors affecting the reproducibility of the method will be investigated on normal volunteers, under controlled dietary conditions. The dietary requirements will be assessed using an Oxylog measurement of oxygen consumption. b) Alterations in protein turnover in patients with malignant disease are studied in inpatients on a controlled diet matched to their measured oxygen consumption. The duration of the study period varies between 3 and 14 days. The longer study period is used only in patients undergoing hyperalimentation. Some patients are studied for a second period either in the immediate post-operative period or at an interval after surgery. In order to attempt to assess protein metabolism in terms of variations in synthesis and breakdown a measurement of protein turnover must be made. Until recently the methodology employed was cumbersome, and the use of C14 prevented serial studies. The method involves a tracer dose of N15 glycine given by mouth, and the cumulative N15 enrichment of urinary ammonia is measured, as is total urinary ammonia excretion over a 12-hour period. This method is non-invasive and requires less patient cooperation than other methods. This study demonstrated a reproducibility of plus or minus 13%. If we are able to show an equivalent or improved reproducibility, this method would be a useful additional means of evaluating protein metabolism in clinical studies.

PROGRESS: Initial control studies using the N15 glycine/12-hour ammonia method showed wide variation, unsuitable for clinical studies. We are therefore undertaking a series of control studies to establish if it is possible, by applying more stringent experimental conditions, including total dietary control, to standardize physical activities during the experimental period to improve the reproducibility of this method.

332. MOLECULAR EVENTS DURING TRANSITIONS IN GROWTH RATE

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Increased production of new ribosome particles is a conspicuous event following initiation of an increased growth rate. These studies are designed to study the control of synthesis of eucaryotic ribosome particles at the transcriptional and translational levels. Particular attention will be paid to structural ribosomal proteins (r proteins) employing two model systems - (1) regenerating rat liver, and (2) tissue culture cells submitted to growth stimulation. The former will be used to isolate messenger RNA molecules for r proteins since early during regeneration their production is exaggerated. We shall isolate mRNA from the light fraction of polyribosomes because they are believed to contain messages for structural r proteins. At various stages, mRNAs will be translated in a wheat germ system to assess the degree of purity and enrichment of messages for proteins. Isolation of an mRNA molecule for one or more individual r proteins would permit synthesis of a labelled cDNA molecule. Availability of this probe would allow assessment of the number of genes for r proteins and of transcriptional events which occur during transitions in growth rate. Studies using tissue culture (3T3) cells will allow us to follow synthesis of individual proteins in intact cells early during perturbation of the growth rate. Labelled cDNA isolated above will be used to monitor transcriptional events and to provide information concerning the involvement of mRNP complexes in the expression of genes for proteins. These investigations should provide an insight into the regulation of r protein synthesis and greater understanding of the growth process.

333. INTERMEDIARY METABOLISM IN ANIMALS AND IN MAN

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The goal of this work is the elucidation of the mechanisms controlling the rate of protein synthesis at the cytoplasmic level in mammalian cells, and the differences between these mechanisms in normal and malignant cells. The tissue utilized in the present studies is the Ehrlich ascites tumor cell growing in suspension culture. This cell demonstrates cytoplasmic control mechanisms which respond vigorously to alterations in nutrient (essential amino acid, glucose, or serum) concentration in the medium. We have demonstrated that upon nutrient deprival one such mechanism operates to inhibit polypeptide chain initiation by inhibiting the binding of Met-tRNAf to the native 40s ribosomal subunit. This step requires eucaryotic initiation factor 2 (eIF-2), which, together with Met-tRNAf and GTP form a ternary complex. We are presently examining factors affecting ternary complex formation. We find a soluble inhibitor of complex formation, and a soluble protein which prevents this inhibition. We are comparing the affects of these factors upon phosphorylated and unphosphorylated eIF-2. We find an eIF-2 kinase and an eIF-2 phosphatase, and are studying the extent of phosphorylation of ribosome-bound and free eIF-2 in fed and nutritionally deprived cells.

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334. STUDY OF ABNORMAL PROTEIN DEGRADATION IN E. COLI

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The long-term objective of the proposed research is the isolation and characterization of the ATP-dependent protein degradation system of E. coli responsible for the catabolism of abnormal proteins. Once the homogeneous system is isolated, characterization will be directed towards: (1) substrate specificity, (2) the mechanism of energy coupling, (3) factors determining the regulation of the system.

The proposed approach for the attainment of these objectives can be summarized as follows: (1) The isolation of the degradative systems will be attempted using classical protein purification techniques. (2) Substrate specificity will be studied by monitoring the catabolism of peptides of known primary and tertiary structure. (3) ATP-dependence will be studied through the use of radiolabelled ATP. ATPase activity will be assayed in the native degradative system to determine whether phosphorylation, adenylation or adenylylation occurs. Attempts will be made to dissociate ATPase activity from proteolytic activity. If the activity is dissociable, both ATPase and proteolytic activity will be studied independently. (4) The factors that determine the regulation of the system will be studied by immunological and activity assays.

My current plans are to develop a model system for the study of protein degradation under pathological conditions in mammals using the approaches described above.

335. GROWTH AND DIFFERENTIATED FUNCTION OF KERATINOCYTES

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The multiplication and differentiated behavior of the keratinocyte of stratified squamous epithelia will be examined in detail. Cultures of this cell type contain multiplying and terminally differentiating cells. The control of the transition of the cells from proliferative to terminally differentiating phases will be examined. The principal differentiated products of this cell type are the keratins and the cross-linked envelope. The synthesis of these products is regulated according to the stage of terminal differentiation, which is defined by the position of the cells in the natural epithelium or by the size of the cells. As the cells differentiate they change the keratins they synthesize, and begin the synthesis of new proteins (such as the envelope precursor) not synthesized in the cells of the basal layer. To a considerable extent, these changes also take place in cultured keratinocytes. The control of this process will be studed as a manifestation of controlled gene expression.

336. MECHANISM OF TRANSLATIONAL CONTROL IN MAMMALIAN CELLS

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Even though much of the biochemistry of protein synthesis in mammalian cells has been worked out, relatively little is known about how translation is controlled and whether the mechanism(s) of this control is the same for all types of cells in all types of situations. This application describes two experimental systems which should provide information relevant to these questions. We have shown that several inhibitors of RNA synthesis - actinomycin D, MPB, and cordycepin -unexpectedly inhibit translation in intact mouse L-cells, but have no effect on rabbit reticulocytes or L-cell lysates. We propose to determine the mechanism of this effect by systematically analyzing the characteristics of lysates from treated cells (whose activity is inhibited) and determining the nature and cause of the translational lesion(s). The second part of this proposal deals with the analysis of the role of glutathione (GSH) in regulating protein synthesis. The GSH system has been studied, so far, only in reticulocytes, and it is not yet clear whether these results are applicable to other cells which are not terminally differentiated. Our preliminary results suggest that translation in L-cells may also be influenced by the level of oxidized GSH, and so experiments are proposed to follow up these observations in cell-free systems and to determine the mechanism. Because of the vital role of translational control in the regulation of gene expression, the results of these experiments should be important in increasing our knowledge of the mechanism regulating the growth, function, and differentiation of both normal and diseased cells.

337. FROM GENE TO PROTEIN - STRUCTURE FUNCTION AND CONTROL IN EUKARYOTIC CELLS

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The production of human lymphoblastoid interferon messenger RNA synthesis was characterized in Namalva cells after induction with Newcastle disease virus. The level of interferon mRNA increased 50- to 100-fold within 8 hours of induction. The amount remained constant for an additional 6 hours but began to decrease by 17 hours postinduction. Under the latter conditions the entire cellular complement of interferon mRNA was found free in the cytoplasm. In contrast, 50% of the interferon mRNA was polysome-associated earlier, during active synthesis of the antiviral protein. At both times interferon mRNA was fully translatable in oocytes of Xenopus laevis. Since interferon protein is virtually fully induced 11 hours after the initiation of virus treatment, the shut-off of interferon synthesis cannot be attributed to decay of its mRNA. These results show that control of interferon synthesis in Namalva cells is a function of mRNA translatability, not of mRNA stability. The data explain, in part, why superinduction of interferon mRNA has never been observed in these cells. Immune interferon mRNA was isolated from human lymphocytes stimulated with staphylococcal enterotoxin A. The yield is approximately 200-fold lower than the yield of lymphoblastoid interferon mRNA from Namalva cells.

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338. HORMONAL MECHANISMS REGULATING EXPRESSION OF MILK PROTEINS IN RAT MAMMARY GLAND AND RAT MAMMARY TUMORS (ABBREV)

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Rat alpha-LA cDNA and casein cDNA clones have been constructed. Alpha-LA cDNA clone carrying 350 base pair sequence of rat alpha-LA mRNA has been characterized. Using this clone, studies of the organization and the state of methylation of alpha-LA gene sequences during the functional differentiation of rat mammary gland and in various mammary tumors have shown: a) inverse relationship between the expression of the alpha-LA gene and its methylation and b) alpha-LA gene sequences or part of the sequences are reiterated in MTW9 tumor DNA.

Analysis of the in vitro synthesized translational products programmed with the poly A plus RNA from the mammary gland and tumors have shown: a) changes in the mRNA activities occurring during the functional differentiation of the mammary gland; b) larger alpha-LA is expressed during pregnancy and both small and large alpha-LA are expressed during lacta-

tion and c) MTW9 and MccLx tumor RNA activities are comparable with the gland at early stages of pregnancy except lower alpha-LA mRNA activities are present in these tumors.

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339. ORGANIZATION AND CONTROL OF GENETIC MATERIAL IN IMMUNOGLOBULIN-SECRETING PLASMACYTOMAS

Mushinski, J. F., Potter, M., Hatfield, D., Fitzmaurice, L., Tucker, P. W., Blattner, F. R., Filkelman, F. D., Kessler, S. W., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Immunochemistry Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

It is the long range purpose of this project to study the control mechanisms important in regulating protein synthesis in normal and malignant cells. Th organization of immunoglobulin light and heavy chain genes and the control mechanisms active in selecting those being transcribed and those which are expressed in protein synthesis are being studied. Particular attention has been devoted to the molecular biology of mouse IgD since our identification during the past year of the first two mouse plasmacytomas that secrete this class of Ig. The nature of the two IgD myeloma proteins, their glycosylation during biosynthesis and secretion, and their antigenic properties have been investigated. mRNA for the IgD heavy chain has been prepared from one tumor and used to synthesize cDNA. This cDNA has been cloned in bacteria and used to isolate a genomic DNA clone containing the IgD heavy chain. The DNA sequence of the cDNA clone and the genomic clone have been partially completed. The IgD heavy chain constant region gene has been found to be close to the IgM constant region gene in mouse liver DNA. The transcription of these genes is being studied.

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340. BIOCHEMISTRY OF EARLY MAMMALIAN DEVELOPMENT

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An investigation of protein syntheses during oogenesis and the preimplantation period of embryonic development will be undertaken. Particular emphasis will be placed on gene expression and control and on the synthesis of three classes of proteins: nuclear proteins (histones and non-histone chromosomal proteins), cell surface proteins, and mitochondrial proteins. Nuclear proteins and mitochondrial proteins will be analyzed at various developmental stages after radioactive labeling and cell fractionation. Cell surface protein will be either labeled endogeneously and isolated by precipitation with lectins or will be labeled by exogenous surface labeling techniques. With these studies as a foundation, three types of lethal mutations affecting preimplantation development will be analyzed. In each case, identical-twin 'half embryos' will be prepared to permit definitive identification of the mutant homozygotes, and appropriate translocation chromosome markers will be used to facilitate identification. The mutations to be studied as three in the T-complex (t12, tw32, Thp) yellow (Ay), and oligosyndactyly (Os). The investigations of the T-locus mutants and Ay will emphasize surface proteins and metabolic regulation, as well as other aspects of protein and lipid synthesis. The study of Os will be directed toward an analysis of the synthesis and structure of tubulin and other microtubule proteins.

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341. ECTOPIC PLACENTAL PROTEINS IN CANCER

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The general problem with which we are concerned is the elucidation of cellular mechanisms of gene regulation which are related to the neoplastic process in humans. The phenomenon of ectopic protein synthesis in human cancer offers a good experimental model for investigating this problem. The ectopic synthesis of placenta proteins by non-trophoblastic neoplasms is of special interest because of the frequent association of similar characteristics in neoplastic cells and embryonic cells. This has suggested that there may be a link between the mechanisms associated with the de-repression of the genes synthesizing embryonic proteins and those involved in neoplastic transformation.

We are conducting experiments in two fundamental categories: 1) Studies on the regulation of gene expression in normal and neoplastic cells. In these studies we measure and compare patterns of synthesis of placental proteins and their corresponding specific messengers in normal trophoblast, in choriocarcinoma, and in non-trophoblastic tumors to determine whether similar regulatory mechanisms are operant. These experiments are performed using tissue cultures derived from trophoblasts and from tumors which synthesize placental proteins ectopically. 2) Studies on the structure, function, and tissue distribution of trophoblastic plasma membrane glycoproteins. These studies are a continuation of the characterization of glycoproteins expressed on the brush border membrane of the term placenta. We will determine the distribution of these proteins among various normal adult tissues and a variety of tumor tissues. Furthermore, we will attempt to identify specific functions with individual glycoprotein components and study their topographical distribution on cells in different physiological states.

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342. INHIBITION OF PROTEINS BIOSYNTHESIS BY CATABOLIZING ENZYME (MICE, RATS)

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This is part of a broader project. A summary of this subproject is not available.

C. RIBOSOMAL FACTORS IN TRANSLATIONAL CONTROL OF PROTEIN SYNTHESIS AND RELATED STUDIES

343. PHOSPHORYLATION OF RIBOSOMAL PROTEINS AND THE CONTROL OF MAMMALIAN PROTEIN SYNTHESIS

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The objective of this project is to develop cell-free protein synthesizing systems from mouse myeloma cells grown in culture which show high activity, comparable with that of reticulocyte lysates. The aim of this work is to deepen our understanding of the factors which regulate protein synthesis in normal and neoplastic cells. Previous work with cell-free extracts of mouse myeloma cells has shown that they are capable of synthesizing protein at a high rate for only a very short time, after which the initiation of new polypeptide chains is severely impaired. In trying to overcome this defect, we have adopted two kinds of approaches. First, we will analyze the precise site of

the lesion and try to determine whether inhibition is due to the activation of specific inhibitory regulators of protein synthesis. Secondly, we are trying to discover what factors may be added to the cell-free systems in order to overcome the inhibition. We are particularly concerned with testing the hypothesis that the state of phosphorylation of the 40S ribosomal protein S6 may determine the rate of protein synthesis in these cells, since recent evidence suggests that the degree of phosphorylation of this protein may be correlated with the rate of protein synthesis. Such evidence has been obtained from studies on intact cells, and it is most important to determine whether deliberate alterations to the phosphorylation of S6 have any effect on the rate of protein synthesis in cell-free extracts.

We have determined that the major site of inhibition of protein synthesis in myeloma cell-free systems is at the level of binding messenger RNA to ribosomes. We have also found that addition of ribosome-free supernatant from rabbit reticulocytes contains a factor or factors which reliably stimulate protein synthesis in these extracts. We are trying to identify this factor in order to determine its mode of action.

344. PROTEINS OF RNA TUMOR VIRUSES - SYNTHESIS AND MATURATION

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OBJECTIVE: Studies on the maturation of MuLV virion proteins at the plasma membrane of chronically infected cells. The ts-3 mutant of Moloney leukemia virus which is temperature sensitive for virus budding also fails to process precursor gag and env proteins at the non-permissive temperature. Coinfection of these cells with the cytolytic enveloped RNA virus of vesicular stomatitis prevents the release of transcriptase-active ts-3 particles normally observed upon shift-down to the permissive temperature. The system is being used to determine the nature of the block on virus release.

Preliminary studies indicate that VSV superinfection inhibits the normal appearance of p30 product upon shift-down. Current experiments examine this process in more detail, attempting to define the stage of inhibition of processing and the VSV functions required to produce this effect.

345. MEMBRANE-ASSOCIATED RIBOSOMES AND PROTEIN SECRETION IN BACTERIA

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We have developed a method for extracellular labeling of growing peptide chains in bacteria, and have thereby unequivocally demonstrated the secretion of a growing chain across a membrane. We wish to use this method, and also to observe synthesis by membrane-associated polysomes or by free polysomes in vitro, to determine how generally the synthesis of secreted proteins (including bacterial toxins), and the insertion of membrane proteins, proceeds in this manner.

The role of the leader sequence and a possible membrane receptor, in the binding of ribosomes (via their nascent chain) to membrane, will be studied by reconstituting membrane-polysome complexes in vitro from polysomes and membranes. We will use homologous and heterologous membranes; synthetic liposomes containing various membrane proteins; nascent chains with modified leaders; and identification, by cross-linking and by inhibition of chemical derivatization, of membrane proteins that are adjacent to ribosome proteins (and vice versa).

The most challenging question - the mechanism of transport of the chain across the membrane - will be studied by the use of mutants altered in secretion, cells with altered lipid composition, a search for an energy requirement, and cross-linking of protruding growing chains to adjacent membrane proteins.

Finally, we shall seek an explanation for the surprisingly large fraction of ribosomes (ca. 50%) with an apparently physiological attachment to membrane.

346. MECHANISM OF ACTION OF INITIATION FACTORS IN BACTERIAL PROTEIN SYNTHESIS

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Translational controls most likely operate during the initiation phase of protein synthesis. The proposed projects are designed to elucidate the mechanism of action of initiation factors and to determine whether they play a modulatory role during initiation in the bacterium, Escherichia coli. Advantage will be taken of the availability, in this laboratory, of pure initiation factors, specific antibodies against them, and the knowledge and technology required for the identification of all the ribosomal proteins. First, the expression of initiation factor genes will be studied and the effects on protein synthesis of changing intracellular levels of the factors will be determined. Factor concentrations will be measured by a quantitative, sensitive radioimmunoassay in lysates of cells harvested in different physiological states. The rates of initiation factor synthesis and turnover relative to that of total cell protein will be determined by using the antibodies to purify pulse-labeled factors. Changes caused by nutritional shift-up, amino acid starvation growth rate and viral infection will be studied. Second, current studies on the structural elements of ribosomes which are directly concerned with the initiation process will be extended. Crosslinking with cleavable bifunctional reagents of initiation factors complexed to ribosomes will allow the identification of ribosomal proteins in direct contact with the factors and thus a definition of ribosomal binding sites. Third, a study of the kinetics of initiation factor-ribosome interactions is proposed which utilizes fluorescence polarization techniques. This work should contribute to our understanding of the pathway of initiation and should lead to the identification of the rate limiting step. The projects entail the application of new experimental techniques, and will help to elucidate the nature of the fundamental mechanisms of protein synthesis in bacteria.

347. SPATIAL ARRANGEMENT OF EUCARYOTIC RIBOSOMAL PROTEINS

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The eucaryotic ribosome is larger and more complex than that of Escherichia coli. There are more proteins (70 vs 55) of greater average molecular weight. Reversible protein:protein crosslinking with the reagent 2-iminothiolane (methyl 4-mercaptobutyr-imidate) and diagonal polyacrylamide/dodecyl sulfate gel electrophoresis have been used previously in this laboratory to identify thirty-five crosslinked protein pairs in the small bacterial ribosomal subunit and twenty-five in the large subunit. The same techniques will be applied to ribosomal subunits obtained from a eucaryotic source: rabbit reticulocytes. The information thus obtained will enable construction of rough maps of the topography of the ribosomal proteins. The information should supplement other structural and functional data. Particular attention will be focused upon sites of functional importance: the initiation site identified by crosslinking complexes of ribosomes and pure initiation factors; the interface between the two subunits; the peptidyl transferase site; the site of a particular phosphoprotein in the small subunit. The structural information obtained should aid in providing a framework for understanding the mechanism and control of protein synthesis in higher organisms.

348. COMPARATIVE STRUCTURE AND FUNCTION OF RIBOSOMES

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The objective of these studies is to determine the relationship between the structure and function of the ribosome in normal and cancerous eucaryotic cells. We will study the role of the ribosome in the following processes: protein synthesis, the control of protein synthesis, RNA synthesis, and the control of RNA synthesis in normal and cancerous eucaryotic cells to determine if there are any differences in these processes between normal and cancerous cells. Nutritional deprivation in conjunction with inhibitors and mutants that affect RNA and protein synthesis will be used to study the control mechanisms of RNA and protein synthesis. These studies will further define the precise mechanisms of RNA and protein metabolism in eucaryotic

cells and will lead to a precise understanding of the mechanisms of action of the ribosome.

The role of the ribosome in RNA and protein synthesis in conditional lethal mutants of eucaryotic cells will be analyzed at the cellular level, in vivo, by the following biochemical-physiological tests that we have developed or refined: incorporation of labelled precursors into DNA, RNA, protein and polysaccharide, polysome stability, and pulse-labelled RNA and protein entry into polysomes and other cell components. Differences in RNA and protein metabolism will be studied in vitro by biochemical tests we have developed to characterize conditional lethal mutants defective in protein synthesis in yeast. This approach - first in vivo tests, then specific in vitro tests - will also enable us to determine the precise mechanisms of action of antitumor agents that affect RNA and protein synthesis. Conditional lethal mutants of mammalian cells will be isolated and analyzed to locate their precise defect.

These tests will be applied to the following mammalian cell lines: mouse BALB/3T3 cells, Hamster CHO and human HeLa cells; and to the eucaryotic organism, Saccharomyces cerevisiae. Mutant isolation will be restricted to the CHO cell lines and S. cerevisiae.

349. BIOCHEMICAL CHANGES RELATED TO ONSET AND CESSATION OF NORMAL CELL GROWTH

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OBJECTIVES: To characterize the behavior of ribosomal particles in a non-growing cell population in which the rate of protein synthesis is maintained at low levels; to correlate such behavior with the limitation in protein synthesis so as to understand the mode of translational control employed by the cell in adjusting protein synthesis to growth and metabolic activities; to determine the regulatory steps which are operative in the enhancement of protein synthesis associated with the onset of cell growth; to relate modifications of the cell membrane with biochemical alterations regulating nuclear and cytoplasmic functions as they participate in control of cell proliferation; to detect and characterize the biochemical steps which control protein synthesis during the physiological cessation of cell growth and division.

BIBLIOGRAPHIC REFERENCES: Folk, J., Park, M-H., Chung, S., Schrode, J., Lester, E. and Cooper, H. Polyamines as physiological substrates for transglutaminases. J. Biol. Chem. 255: 3695-3700, 1980. Cooper, H.L. Evidence for a non-messenger RNA which is rate-limiting for protein synthesis in lymphocytes. In Quastel, M. (Ed.): Cell Biology and Immunology of Immune Function. New York, Academic Press, 1979, pp. 135-142.

350. INHIBITORS OF RIBOSOMAL FUNCTIONS

Vince, R., University of Minnesota, Minneapolis Campus, School of Pharmacy, Dept. of Medicinal Chemistry, *Morrill Hall, Minneapolis, Minnesota*, 55455, United States of America

Our main objective is to prepare inhibitors of protein biosynthesis and to study the mechanism of peptide bond formation at the ribosomal level. We have previously completed an extensive study of the ribosome peptidyl transferase center using model substrates and inhibitors of this peptide-forming structure. We plan to extend these studies to find different binding requirements among ribosomes from different sources which will enable us to prepare selective inhibitors of protein synthesis. These agents will have potential as chemotherapeutic agents.

BIBLIOGRAPHIC REFERENCES: Poly(2-fluoradenylic acid). The Role of Basicity in the Stabilization of Complementary Helices, Arthur D. Broom, Venkataraman Amarnath, Jay Brownell and Robert Vince, Biochim. Biophys. Acta., 563, 508-517 (1979). Facile Synthesis of Carbocyclic Lyxo- and Ribonucleosides, Robert Vince and Susan Daluge, J. Org. Chem., 45, 531-533 (1980).

351. RIBOSOME STRUCTURE AND FUNCTION

Kurland, C. G., Uppsala Universitet, Molecular Biology Inst., *Box 562, 75122 Uppsala, Sweden*

The ribosome as the site of protein biosynthesis plays a central role in normal and pathological cell activities. Our studies of this universal organelle are directed at the mechanisms of ribosome function as well as the physiological control of its activities. The current focus of our studies concerns the molecular mechanisms and cellular strategies that determine the accuracy of translation.

We have developed a unique in vitro system that supports translation at missense error frequencies in the same range as those observed in vivo. We have used this system to demonstrate the critical role played by the GTP fluxes over the ribosome in determining the accuracy of translation. A principal objective of our future studies is to understand the mechanism of this effect. Similarly, we have simulated in vitro the effects on translation of the physiological effectors known as 'magic spots'. Understanding the mechanism of their action is another of our principal objectives.

There are at present no data for specific codon and context dependencies of the errors of translation. We are developing a technology to obtain such data in vivo in order to understand why different cell types have different codon preferences.

In collaboration with others we will continue our studies of the kinetics of translation, of the molecular genetics of ribosomes and factors, as well as of the crystallographic structures of components of the translation system.

352. MECHANISM AND REGULATION OF RIBOSOMAL REACTIONS

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No summary has been provided to the Smithsonian Science Information Exchange.

353. RIBOSOMAL PROTEINS

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This is part of a broader project. A summary of this subproject is not available.

IV. BIOCHEMISTRY OF AMINO ACIDS AND POLYAMINES

A. BIOCHEMISTRY OF AMINO ACIDS

354. AMINO ACID X - STRUCTURE, DISTRIBUTION, BIOSYNTHESIS, AND FUNCTION

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Elongation factor 2 (EF-2), the protein which promotes the translocation step during protein synthesis in the cytoplasm of eukaryotic cells, contains an unusual amino acid, designated X by Robinson, Hendriksen, and Maxwell (J. Biol. Chem. 249, 5088, 1974). Amino acid X appears to be essential to the function of EF-2 because its covalent modification by diphtheria toxin inactivates the protein. Although the toxin will apparently inactivate all eukaryotic EF-2s through this modification, it does not appear to modify any other protein. This suggests that all EF-2s contain a functionally essential and unique structural feature which is specifically recognized by diphtheria toxin. The toxin inactivates EF-2 by transferring the ADP-ribose of NAD(plus) to the unusual amino acid X. Amino acid X almost certainly arises by post-translational modification of a precursor of EF-2 because it is not one of the 20 common amino acids. Interestingly, it also appears to differ from the previously recognized amino acids known to arise by post-translational modification of proteins.

We have developed a large scale method for the purification of amino acid X from yeast EF-2. The initial goal of this proposal is to utilize the material we have prepared by this method to determine the structure of amino acid X and the mode of ADP ribose attachment. For this purpose we will employ primarily nuclear magnetic resonance spectroscopy and mass spectroscopy. Immediately following the determination of the structure of amino acid X we propose to initiate studies designed to determine: 1) The distribution of amino acid X in

other proteins of various biological materials; 2) the mechanism of biosynthesis of amino acid X; 3) the role of amino acid X in the function of EF-2; 4) the nature of the diphtheria toxin recognition site on EF-2.

355. METABOLISM OF AROMATIC AMINO ACIDS

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The enzymes that catalyze the synthesis and degradation of the aromatic amino acids and various related aromatic compounds are particularly suitable for detailed biochemical analysis. The mechanisms by which the reactions are carried out and the means by which various organisms regulate these complex biosynthetic and degradative processes are of considerable fundamental interest. At the same time, a knowledge of the biochemistry of these multienzyme systems can be of immediate practical importance. For example, malfunction of the enzymes which catabolize the aromatic amino acids has been implicated in a variety of diseases such as schizophrenia, Parkinson's disease, phenylketonuria, bladder cancer, arteriosclerosis and heart failure. Moreover, numerous aromatic compounds in our environment have been shown to be hazardous to man, and it is known that mammals have enzymes which can activate or inactivate these substances. In addition, the aromatic amino acid tryptophan has been implicated as a possible intermediary of genetic damage by ultraviolet radiation, and serotonin, a metabolite of tryptophan, may be a radioprotectant. The genetics of the enzymes which synthesize the aromatic amino acids in eukaryotes are of special interest and have been especially well characterized in Neurospora crassa. Moreover, we have begun to establish genetic markers for the enzymes which catabolize tryptophan in mouse. Obviously, the scope of the present proposal could not begin to address all of the possibilities noted above. However, the above problems illustrate the wide range of fundamental and practical issues to which the proposed research may contribute.

BIBLIOGRAPHIC REFERENCES: Gaertner, F.H., et al, The Arom Enzyme Conjugate of Neurospora: Multiple Proteases and Subunit Artifacts, pp. 197-202 in Limited Proteolysis in Microorganisms, G.N. Cohem and H. Holzer (eds.), Library of Congress, NIH, 1979. Vitto, A., and F.H. Gaertner, Proteolytic Inactivation and Coordinate Protection of the Arom Enzyme Conjugate of Neurospora, pp. 203-207 in Limited Proteolysis in Microoganisms, G.N. Cohen and H. Holzer (eds.), Library of Congress, NIH, 1979.

356. REGULATION OF TYROSINE SYNTHESIS IN HEPATOMA CELLS

Shiman, R., Pennsylvania State University, Milton S. Hershey Medical Center, School of Medicine, Dept. of Biological Chemistry, *500 University Dr., Hershey, Pennsylvania*, 17033, United States of America

We will investigate the regulation of tyrosine synthesis in rat hepatoma cells growing in culture. Both metabolic control (involving effects of substrate and product levels, and membrane transport properties) and genetic control (involving effects on protein synthesis and degradation) of the rate of hydroxylation of phenylalanine to tyrosine will be examined. The mechanisms of regulation of phenylalanine hydroxylase by its substrate phenylalanine, by hormones and by cell culture population density will be studied in detail. In this latter case, we want to establish the nature of the cell-cell interactions involved in affecting tyrosine synthesis as well as the nature of the intracellular controls mediating the process. Primary rat liver cells as well as the hepatoma cells will be examined, both for an insight into the regulatory process in two different, but related, systems and into differences between the normal and transformed cells.

Biochemical methods are being applied to a basic problem relating to metabolism of normal and tumor cells and to the biology and control of a complete enzyme system. This system is required for normal animal development (it is defective in the human disease phenylketonuria), and provides a good model system for understanding the regulation of cell growth and division by intercellular interactions among mammalia cells.

357. CONTROL MECHANISMS IN PRO- AND EUCARYOTIC CELLS

Pizer, L. I., Yoshikawa, M., University of Colorado, Health Sciences Center, School of Medicine, Dept. of Microbiology & Immunology, *4200 E. 9th Ave., Denver, Colorado*, 80262, United States of America

We propose to study the regulation of serine and glycine biosynthesis in bacterial and mammalian cells, with the object of defining and understanding the mechanisms which control metabolism. The bacterial system is controlled by end product inhibition of enzyme activity. The responsible enzyme has been crystallized, and the mechanism of serine inhibition will be investigated at the chemical level in order to elucidate how small molecules influence protein structure. The basis for the constancy in enzyme levels in bacteria will be investigated by combining genetic and enzymological techniques. Mammalian cells regulate the production or stability of the enzymes responsible for serine biosynthesis. The fluctuations that occur in the levels of the enzymes responsible for serine biosynthesis in mammalian cells will be investigated to determine whether the rates of synthesis or degradation are affected. The enzymes responsible for the production and degradation of glycine also change activity with growth conditions. The compounds responsible for the alterations in these enzymatic systems will be identified and the mechanisms by which they interact with the processing of macromolecules determined. The ultimate aim of this project is to understand how cells maintain balanced growth and to explain how aberrant regulatory processes cause pathological and malignant conditions.

BIBLIOGRAPHIC REFERENCES: Pfendner, W., and Pizer, L.I. The Metabolism of Serine and Glycine in Mutant Lines of Chinese Hamster Ovary Cells. Archives of Biochem. and Biophys. Vol. 200, pp. 508-512 (1980). McKitrick, J.C., Pizer, L.I. Regulation of Phosphoglycerate Dehydrogenase Levels and Effect on Serine Synthesis in Escherichia coli K-12. J. Bacteriol. Vol. 141, pp. 235-245 (1980).

358. REGULATION OF ONE CARBON METABOLISM

Stauffer, G. V., University of Iowa, School of Medicine, Dept. of Microbiology, *Jessup Hall, Iowa City, Iowa*, 52240, United States of America

The overall objective is to define the molecular mechanism(s) that regulate glycine synthesis and the flow of one carbon units in Salmonella typhimurium and Escherichia coli. Regulatory mutants resistant to specific amino acid analogs will be isolated. In addition, the lac structural genes have been fused to the promotor controlling glyA gene expression as a means of isolating and studying mutants with altered control of the glycine pathway. Genetic techniques (conjugation, transduction) will be used to locate the sites of these mutations on the E. coli and S. typhimurium linkage map. Physiological studies will be performed to determine the consequences of these mutations in regulation and to determine the different regulatory components involved in the control mechanism(s). Finally, the glyA gene and its controlling segment from S. typhimurium and E. coli will be cloned into plasmid vehicles, and restriction mapping and DNA sequencing techniques will be used to determine the location and nucleotide sequence of the controlling segment for the glyA gene. The responses of the control segment in vitro to the addition of the components found to be important for regulation during the physiological experiments will be examined.

359. STUDIES OF AMINO ACID BIOSYNTHESIS AND DEGRADATION

Phang, J. M., Yeh, G. C., Williams, J. A., Hagedorn, C. H., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Endocrinology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The enzymes catalyzing the interconversion of proline and pyrroline-5-carboxylate provide a mechanism for the intercompartmental intercellular and interorgan transfer of redox potential. The proline cycle links glucose oxidation through the hexosemonophosphate-pentose pathway to mitochondrial oxidative phosphorylation. Ornithine and glutamate can serve as additional sources of pyrroline-5-carboxylate.

BIBLIOGRAPHIC REFERENCES: Phang, J.M., Downing, S.J., and Yeh, G.C.: Linkage of the HMP pathway to ATP generation by the proline cycle. Biochem. Biophys. Res. Commun. 93: 462-470, 1980. Yeh, G.C., and Phang, J.M.: The function of pyrroline-5-Carboxylate reductase in human erythrocytes. Biochem. Biophys. Res. Commun. 94: 450-457, 1980.

360. THE METABOLISM OF HYDROXYPROLINE IN WOUNDED TISSUE

Varner, J. E., Washington University, School of Arts & Sciences, Dept. of Biology, *4559 Scott Ave., St. Louis, Missouri*, 63130, United States of America

Wounded plant tissues develop the capacity to synthesize and secrete large quantities of hydroxyproline-rich glycoproteins. At the same time they develop a marked capacity to convert free hydroxyproline directly to proline. This proline is reutilized to make proteins, including hydroxyproline-rich glycoproteins.

During my sabbatical leave I plan to 1) determine the intermediates in this path for the conversion of hydroxyproline to proline, 2) isolate and characterize the enzymes that catalyze the reactions in this path, 3) look for the role of this path in normal and in wounded tissue.

361. HEPATIC SYNTHESIS AND RELEASE OF TRIGLYCERIDE

Waterhouse, C., University of Rochester, School of Medicine & Dentistry, Dept. of Medicine, *601 Elmwood Ave., P.O. Box 607, Rochester, New York*, 14642, United States of America

The proposed work is designed to study amino acid metabolism, with reference to turnover, oxidation and incorporation into serum proteins in patients who have progressive cancer with weight loss. The technique would be the administration of 14C labelled amino acids with determination of radioactivity in the amino acid given, other amino acids, glucose, CO2 of expired air and serum proteins. The data analysis involves renewal theory and circulating flow rates. The purpose of the study is to determine whether there are changes in amino acid catabolism and rate of synthesis into serum protein in this group of patients.

362. METABOLIC ACTIVATION AND INACTIVATION OF AMINO ACID PYROLYSATE BY PEROXIDASE

Yamada, M., Ministry of Health & Welfare, Biochemistry Div., *5-1-1 Tsukiji, Chuo-ku, Tokyo, Tokyo, Japan*

OBJECTIVE: Tryptophan pyrolysates are mutagenic to Salmonella typhimurium TA98 by Ames/S-9 mix test and also can induce the transformation of hamster embryo cells and sister chromatic exchanges of human lymphoblastic cells. Therefore, these compounds may play important roles in etiology of human cancer.

We have found that myeloperoxidase and peroxidases from other sources could degrade these mutagens. Peroxidases are ubiquitously present in many tissues of animals. Purpose of the present studies is to clarify the physiological roles of peroxidases in the metabolic activation and inactivation of these mutagens.

APPROACH: 1) Purification of myeloperoxidase. Human promyelocytic leukemia HL-60 cells are good sources for the purification of myeloperoxidase because the content of the enzyme in the cells is high and the cells are easily obtained. 2) Identification of tryptophan pyrolysate products catalyzed by myeloperoxidase. Tryptophan pyrolysate will be converted into several compounds in the presence of H2O2 by myeloperoxidase. These reaction products will be isolated and characterized chemically. The isolated compounds will be tested for DNA-binding ability protein binding ability, and mutagenicity to Salmonella typhimurium TA98.

363. THE EFFECT OF NORHARMAN ON BENZO(A)PYRENE METABOLISM BY RAT LIVER MICROSOMES

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OBJECTIVE: Norharman (which is a tryptophan pyrolysate) showed several kinds of effect on mutagenicity of chemicals, including benzo(a)pyrene. The mechanisms of effect of norharman are to be clarified by metabolic studies.

. APPROACH: The metabolism of benzo(a)pyrene is studied by HPLC analysis. 14C-Benzo(a)pyrene is metabolized with rat liver microsomes in the presence or absence of norharman. The amounts of hydrophilic and hydrophobic metabolites are measured.

PROGRESS: Norharman inhibited the metabolism of parent benzo(a)pyrene. The metabolic steps from hydrophobic metabolites to hydrophilic ones were also inhibited. Thus the amount of hydrophobic metabolites was higher in the presence of norharman than in its absence.

364. CARCINOGENICITY TEST OF PYROLYSIS PRODUCTS OF AMINO ACIDS AND PROTEIN

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OBJECTIVE: In vivo carcinogenicity test of potent mutagenic substances of amino acid and protein pyrolysates.

APPROACH: Mutagenic principles, Trp-P-1 and Trp-P-2 identified in tryptophan pyrolytic product, and tar of albumin pyrolysate have been administered orally to CDF1 mice and Wistar rats, respectively.

PROGRESS: Liver tumors were found in CDF1 mice given Trp-P-1 1 year after the start of experiment.

365. TRYPTOPHAN METABOLITES AND ENZYMES IN CARCINOGENESIS (MICE, INSECTS, BACTERIA)

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This is part of a broader project. A summary of this subproject is not available.

366. THERAPY BY ENZYMIC DEPLETION OF ESSENTIAL AMINO ACIDS

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OBJECTIVES: 1. Purify a sufficiently large quantity of indolyl-3-alkane alpha-hydroxylase for more extensive antineoplastic and biological studies. 2. Evaluate the effect of different treatment schedules with indolyl-3-alkane alpha-hydroxylase on experimental tumors, alone and in combination with selected tryptophan analogues. Included will be studies employing tryptophan analogues which are degraded by indolyl-3-alkane alpha-hydroxylase; these will be administered to tumor-bearing mice in very high doses, followed by rescue with the enzyme. 3. Continue to test known organisms and soil isolates for production of a therapeutically usable tryptophan-degrading enzyme which is highly specific for L-tryptophan. 4. Study tryptophan metabolism in normal and neoplastic cells, including tumors which are known to be associated with excessive serotonin production. 5. Study the mechanism of antitumor activity of enzyme-mediated tryptophan depletion. 6. Study the uptake and metabolic fate of selected tryptophan analogues with normal and neoplastic cells in the presence and absence of exogenous L-tryptophan. 7. Novel bacterial enzymes have been isolated in our laboratory which deplete plasma histidine and lysine when injected in mice. We will further purify and charactize these enzymes and study their effects on growth of experimental tumors, alone and in combination with analogues. 8. Determine the toxicity of new enzymes in animals. 9. Continue our studies concerned with modification of therapeutic enzymes to improve their therapeutic effectiveness (increase biological half-life, block antigenicity, enhance their distribution outside the vascular space to increase effectiveness on solid tumors).

BIBLIOGRAPHIC REFERENCES: Schmer, G. and Roberts, J. Molecular engineering of the antitumor-enzyme indolyl-3-alkane alpha-hydroxylase. Cancer Treat. Rep. 63:1123-1126, 1979. Roberts, J., Schmid, F. A. and Rosenfeld, H. J. Biological and antineoplastic effects of enzyme-mediated in vivo depletion of glutamine, tryptophan and histidine. Cancer Treat. Rep. 63:1045-1054, 1979.

367. PHARMACOLOGIC INTERFERENCE WITH THE METABOLISM OF THE DICARBOXYLIC AMINO ACIDS

Cooney, D. A., Jayaram, H. N., Kensler, T. W., Ardalan, B., Tyagi, A. K., Cysyk, R. L., Huang, C. Y., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Biochemistry Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The mechanism by which analogs and antagonists of the dicarboxylic amino acids and their amides exert toxic and oncolytic effects have been examined with animals, cells in culture, and isolated enzymes. In addition, the mechanisms by which cells become resistant to the toxic or therapeutic actions of these agents have been studied, using resistant variants of transplantable murine tumors growing in vivo and in vitro. In various lines resistance to PALA correlates with elevated levels of L-Aspartic acid Transcarbamylase; however, in at least one resistant variant of the Lewis Lung Carcinoma, resistance is accompanied by increased Carbamyl Phosphate Synthetase II. The locus of action of Acivicin has not yet been characterized, but resistance to this drug is clearly a transport-related phenomenon. In the case of L-Alanosine, resistance has been shown to be due to two factors; 1. an inability to synthesize and/or retain, critical concentrations of the proximate antimetabolite, 'L-Alanosyl-AICOR'; 2. higher basal levels of purine salvage enzymes in the resistant cell.

BIBLIOGRAPHIC REFERENCES: Anandaraj, S., Jayaram, H.N., Cooney, D.A., Tyagi, A.K., Han, N., Thomas, J. and Montgomery, J.: Interaction of L-alanosine (NSC-153353) with enzymes metabolizing L-aspartic acid, L-glutamic acid and their amides. Biochem. Pharmacol. 29: 227-245, 1980. Cooney, D.A., Stergis, G. and Jayaram, H.N.: Enzymes, Therapeutic. Encyclopedia of Chemical Technology. New York, John Wiley and Sons, Inc., 1980, Vol. 9, pp. 225-240.

368. ESSENTIAL AMINO ACID NUTRITION AND MOUSE TUMOR GROWTH

Banks, W. L., Kaplan, A. M., Chan, W. M., Carter, W. H., Elzay, R., Virginia Commonwealth University, School of Basic Sciences & Graduate Studies, Dept. of Biochemistry/Surgery, *901 W. Franklin St., Richmond, Virginia*, 23284, United States of America

The studies proposed in this application are directed toward establishing the nutrient level(s) of essential amino acid(s) that will prevent tumor growth both in vitro and in vivo of four murine tumor models. These include the A26 glioma, Lewis lung carcinoma, MCA 2182 fibrosarcoma and B16 melanoma. By varying the level of each of the nine essential amino acids in the nutrient media, we expect to be able to identify those essential amino acids that are critical for the growth of each model system in vitro. Based upon these findings, we will establish the dietary level of those essential amino acids in vivo that will prevent tumor growth without compromising the host and produce prolonged survival. Moreover, we will determine the effects of nutrient restriction of essential amino acids on their transport and other metabolic parameters in vitro and in vivo. In addition, we will ascertain whether dietary essential amino acid restriction will prevent metastasis. Finally, we will establish whether the dietary essential amino acid restriction will have the same effects on each of several cloned lines of a single methylcholanthrene fibrosarcoma in order to determine whether or not tumor heterogeneity is expressed at the level of amino acid requirements.

369. CYSTINE DEGRADATION FOR CONTROL OF CELL GROWTH

Ressler, C., Yao, K., Neag, P., Hartley, J., University of Connecticut, Health Center, School of Medicine, Dept. of Pharmacology, *Farmington Ave., Farmington, Connecticut*, 06032, United States of America

The aim of the proposed study is to exploit the known specificity of the cystine requirement characteristic of certain human tumor cell lines. Cystine depletion will be correlated with tumor growth in mice inoculated with L-1210 lymphoma and fibrosarcoma cells.

BIBLIOGRAPHIC REFERENCES: 2,5-Dihydrophenylalanine as a Probe for Aromaticity in Structure-Activity Studies in Peptides. C. Ressler, S.N. Banerjee, M. Tsutsumi, L. Diamond and W.H. Sawyer in Peptides: Structure and Biological Function, E. Gross and J. Meienhofer, Eds. pp. 217-220 (1979). Aminonitriles and Aminothioamides Related to Natural Amino Acids. S.N. Banerjee and C. Ressler. Int. J. Peptide Protein Res. 14 234-246 (1979).

370. CYSTEINE DEPENDENCE AND DEPLETION

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This is part of a broader project. A summary of this subproject is not available.

371. HYDROXYPROLINE IN BONE METASTASIS FROM BREAST CANCER (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

372. PHENYLALANINE IN PREMENOPAUSAL PATIENTS WITH PRIMARY BREAST CANCER

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This is part of a broader project. A summary of this subproject is not available.

B. BIOCHEMISTRY OF POLYAMINES

373. INHIBITORS OF POLYAMINE BIOSYNTHESIS IN NEOPLASTIC GROWTH CONTROL - NEW S-ADENOSYL-METHIONINE AND 5'-METHYLTHIOADENOSINE ANALOGS (ABBREV)

Zappia, V., Carteni Farina, M., Porcelli, M., Oliva, A., Pontoni, G., Della Ragione, F., Caccia Puoti, G., Cocchiara, G., Gragnaniello, V., Raiola, C., Universita degli Studi di Napoli, Fac. di Medicina e Chirurgia, Dept. of Biochemistry, *via Orazio 31, 80100 Naples, Italy*

It is well known that when cell growth is stimulated, a significant increase in the rate of polyamine biosynthesis and in cellular polyamine levels is observable. Furthermore it has been demonstrated by several authors that scientific inhibitors of polyamine biosynthesis, i.e. methyl glyoxal bis(guanylhydrazone), difluoro-methylornithine and 1,3 diaminopropane demonstrate in vitro and in vivo a powerful cytostatic action. In an attempt to obtain new specific inhibitors with antiproliferative effect and low cytotoxicity, the synthesis of several 5'-methylthioadenosine (MTA) analogs, i.e. 5'-methylthioformycin, 5'-methylthio-N6-methyladenosine and 5'-methyl-3-deazoadenosine, has been devised. The importance of MTA in cellular metabolism is suggested by the finding that it inhibits many S-adenosylmethionine-dependent methyl transfer ractions, spermine synthase, the synthesis of alpha-amanitin sensitive RNA in explanted salivary glands of D. melanogaster and mitogen and antigen-stimulated lymphocyte proliferation. The new synthesized analogs will be tested as inhibitors of enzymes related to polyamine biosynthesis and for their effect on cellular proliferation. In addition, studies on the permeability of cell membranes to MTA and its analogs by using human erythrocytes as a model system have been carried out. Preliminary results with methyl labeled MTA indicate that the thioether is actively incorporated into the cells and that the process is consistent with facilitated diffusion mechanism. Analysis of the initial velocity transport data suggests the involvement of a saturable system, as shown by Michaelis-Menten type kinetics and by linearity of the Woolf plot. Furthermore, the transport of MTA and its analogs across erythroleukemic Friend's cells is under investigation.

374. RELATIONSHIP BETWEEN POLYAMINES AND CYCLIC NUCLEOTIDES - CONTROL MECHANISMS OF NORMAL AND NEOPLASTIC GROWTH

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Our previous observations indicate that polyamines, as spermine, spermidine and putrescine, at relatively low concentrations cause the decrease of cellular cAMP content, both in normal and neoplastic cell cultures. Considering the inverse

relationship between cAMP concentration inside the cell and the rate of macromolecular synthesis and cell division, the effect of polyamines is consistent with their observed property to act as promoters of cell growth. Furthermore, results obtained by using specific inhibitors of polyamine synthesis, such as methylglioxal bis(guanylhydrazone) and -methylornithine, clearly indicate that cellular polyamine depletion is associated with cAMP accumulation and with a higher sensitivity of the cell to cAMP-mediated effectors, thus supporting the involvement of these biogenic amines in the control mechanism of cellular cAMP content. Preliminary experiments have indicated that in cultured cells, exogenous polyamines do not affect the release of cAMP but they reduce its intracellular content by increasing the rate of its degradation. Both soluble and particulate activities of cAMP-dependent phosphodiesterase (PDE) are elevated after treatment of the cells with the single amines. In this phase of research we will study the possible mechanism(s) by which polyamines affect PDE activity and in particular whether they exert their effects directly or indirectly through some endogenous factor that can activate PDE, such as calmodulin. Furthermore, since the activity of ornithine decarboxylase, the rate limiting enzyme in polyamine synthesis, is increased after cAMP accumulation, we will clarify whether this event is critical in inducing PDE activity by cAMP.

375. SALIVARY GLANDS, POLYAMINES AND CHEMOTHERAPY

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The possible involvement of polyamines in growth, differentiation and neoplasia has stimulated the study of endogenous levels of these compounds (putrescine, spermidine and spermine) and enzymes responsible for their synthesis (ornithine and S-adenosylmethionine decarboxylases). In particular, reports on increased polyamine levels in rapidly growing and neoplastic tissues have prompted a search both for techniques which might be used diagnostically to detect abnormal polyamine levels and for specific inhibitors of polyamine synthesis.

To date, little is known about the polyamines and their synthesizing enzymes in salivary glands or in saliva. In addition, there is little use of chemotherapy for salivary or oral neoplasias and as a result, little is known about specific cytotoxic drug effects in tissues of the oral cavity or salivary glands.

Polyamine and decarboxylase levels and ultrastructural (EM) characteristics will be studied using mouse salivary glands, both normal and those induced to grow by injecting the mouse with isoproterenol (IPR). IPR-induced salivary growth involves both hypertrophy and hyperplasia and is used as a model growth system. The effect of certain antineoplastic drugs on normal and IPR-induced salivary tissues will be studied by correlating the biochemical and ultrastructural characteristics described above. During the study, correlations will be made between the polyamine and decarboxylase levels in the glands, the state of growth of the glands, and the effects of cytotoxic drugs on both the biochemical parameters and the structure/function relationships as observed microscopically.

It is not known if human saliva contains polyamines or their synthesizing enzymes. Saliva will be analyzed to determine if it contains these components in measurable quantities. If present, studies will be performed to examine the feasibility of using such analyses as a diagnostic method for assessing salivary gland growth and/or function.

376. MAMMALIAN POLYAMINE METABOLISM

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Polyamines are intimately linked to the growth of mammalian cells. Many changes in cellular metabolism induced by hormones and other agents are accompanied by marked increases in the rate of synthesis of putrescine and spermidine brought about by increases in the activities of ornithine decarboxylase and S-adenosylmethionine decarboxylase. The proposed experiments will extend knowledge of these enzymes (which have interesting regulatory properties and very rapid apparent rates of synthesis and degradation). These enzymes will be purified to homogeneity by affinity chromatography on pyridoxamine phosphate and methylglyoxal bis(guanylhydrazone) linked to Sepharose respectively. The purified enzymes will be used to raise specific antibodies to the enzymes. Specific irreversible inhibitors of both enzymes (namely alpha-difluoromethylornithine and 1,1'-((methylethanediylidene) -dinitrilo))- bis(e-amino-guanidine)) will be used to test the role of these enzymes in cell growth and differentiation. Labeled forms of these inhibitors will be used to measure specific binding to the enzyme and the number of enzyme molecules. Polyamines are known to be important in the growth of normal and malignant cells and knowledge of the regulation of polyamine synthesis and the effects of depleting cellular polyamines may enable the development of useful cancer chemotherapeutic agents either from existing inhibitors or by the synthesis of new polyamine antagonists.

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377. BIOSYNTHESIS AND BIOLOGICAL FUNCTION OF POLYAMINES

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The naturally occurring polyamines, putrescine, spermidine and spermine seem to be present universally in living organisms. Although it now appears certain that they are required for optimal rates of growth in microorganisms and animal cells, the precise biological role of these compounds is unknown. Mutants of Escherichia coli have been isolated that require spermidine for optimal growth. The influence of spermidine starvation on the topology of plasmid and chromosomal DNA is being investigated; this will then be related to the known effects of spermidine deficiency on macromolecular synthesis. These in vivo studies with spermidine auxotrophs are being related to the action of polyamines in defined in vitro systems through experiments with a homologous series of spermidine analogs with established biological specificity.

The regulation of polyamine biosynthesis is being studied in mitogen-activated lymphocytes, where large increases in the rates of polyamine synthesis and accumulation are observed. In particular, antibodies have been developed to the enzyme S-adenosyl-methionine decarboxylase and these antisera are being used to study the rates of synthesis and degradation of the enzyme in intact cells. Cell-free translation will be used to assay messenger RNA levels and to test the possibility of translational control in this system.

378. POLYAMINE SYNTHESIS IN NEUROSPORA

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This proposal seeks to define a regulatory circuit which governs the synthesis of the polyamines, putrescine and its derivative, spermidine, in Neurospora crassa. (1) That spermidine negatively controls activity and synthesis of ornithine decarboxylase (ODC), the enzyme responsible for putrescine synthesis; and (2) that control is exerted by the minor fraction of the spermidine pool which is freely diffusible, the rest of the spermidine pool being bound to cell constituents. The research will be directed first to an operational definition of bound spermidine and putrescine by means of in vivo isotopic tracer studies and by means of spermidine binding experiments to cell fractions and to permeabilized cells. The use of mutants now available allows manipulation of the polyamine pools between extremes of deprivation and excess. This in turn allows a test of extractability of polyamines over a range of cellular concentration. The same cells may be monitored, prior to extraction, for the rate of the ODC reaction by measuring accumulation of putrescine and

spermidine by thin-layer chromatography. A second effort seeks to define the mechanisms of ODC regulation through enzymological studies of spermidine inhibition, and through other conditions which alter ODC activity in vivo.

379. POLYAMINES AND CHEMICAL CARCINOGENESIS IN VITRO

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An increase in polyamine biosynthesis is usually associated with increased cellular proliferation, suggesting that altered control of ornithine decarboxylase (ODC) and/or S-adenosylmethionine decarboxylase (SAMD) may account for the abnormal growth patterns of malignant cells. To learn more about the differences between normal and transformed cells in this basic metabolic pathway, the regulation of polyamine biosynthesis will be studied in hamster and mouse embryo cells in culture. The synthesis and accumulation of the polyamines, putrescine, spermidine and spermine, will be compared in normal cells, cells exposed to chemical carcinogens, and malignantly transformed cells. The ODC and SAMD activities and the polyamine concentrations under different culture conditions will be determined for each cell type. The relationship between the induction of ODC and the phenomenon of tumor promotion will also be investigated in vitro. The elucidation of how this critical metabolic pathway is involved in the expression and maintenance of the neoplastic phenotype should lead to rational approaches to the chemotherapeutic control of malignancy.

380. POLYAMINES, POLYAMINE ANTIMETABOLITES AND CELL GROWTH

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The role of the polyamines in cell growth and replication will be investigated by following changes in intracellular levels of the polyamines and in the activities of their biosynthetic enzymes, over the course of the cell cycle. Regulation of polyamine biosynthesis will be studied using inhibitors of nucleic acid and protein synthesis. The effect of polyamine antimetabolites on cell cycle traverse will be studied also. To provide a basis for the rational design of improved polyamine antimetabolites the structural requirements for binding to the active site of ornithine decarboxylase will be investigated. These quantitative structure-activity correlations will examine inhibition of ornithine decarboxylase by a series of structural analogs of ornithine. Finally, the potential applicability of polyamine antimetabolites to the chemotherapy of intracranial tumors will be explored using in vitro and in vivo colony forming efficiency assays and increased life span studies.

381. POLYAMINE METABOLISM IN CANCER AND CANCER CHEMOTHERAPY

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The conjugated polyamines which increase in the urine of cancer patients after successful chemotherapy have been identified as acetylputrescine, N1-acetylspermidine and N8-acetylspermidine. Acetylputrescine concentration reflects the growth fraction of the tumor whereas N1-acetylspermidine and N8-acetylspermidine reflect the tumor cell death fraction in response to therapy. We are in the process of developing a rapid radioenzymatic assay for polyamines in biological fluids that could be set up in clinical laboratories in order to allow oncologists to assess within 24-48 hours the efficacy of ongoing chemotherapy. Work is ongoing to characterize the properties of ornithine decarboxylase, the rate-limiting enzyme in polyamine synthesis, in order to attempt to develop drug therapy regimens to control the activity of this critical growth regulating enzyme.

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382. THE ROLE OF POLYAMINES IN THE DETERMINATION OF DNA TOPOLOGY IN BACTERIA

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OBJECTIVE: To clarify whether the involvement of polyamines in cell growth and proliferation, i.e., the physiology of polyamines, can be explained by a single, specific action on DNA gyrase.

APPROACH: By studying whether certain spermidine homologs show the same specificity in activating purified DNA gyrase as they show in restoring normal rates of growth, replication and macromolecule synthesis to polyamine-depleted E. coli mutants.

V. CARBOHYDRATE METABOLISM IN NORMAL AND NEOPLASTIC CELLS

A. BIOSYNTHESIS AND ROLE OF AMINO SUGARS AND MUCOPOLYSACCHARIDES IN NORMAL AND NEOPLASTIC CELLS

383. ANIMAL CELL SURFACE HEPARIN SULFATE AND TRANSFORMATION

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The ability of cells to respond to each other and the substratum (forms of cell social behavior) is an important part of normal growth and development. Failures in these responses are believed to be involved in metastasis and the loss of contact inhibition of growth, forms of cell social behavior displayed by tumor cells. The mechanism by which cell social behavior is regulated remains unknown.

One common model for the regulation of cell social behavior is that it is mediated by cell surface carbohydrate, in the form of glycolipid and/or glycoprotein. I have proposed that heparan sulfate, a member of the third class of cell surface carbohydrates, the glycoaminoglycans, is involved in the expression of cell social behavior. This proposal is based, in part, upon my observation that there is a decrease in the sulfate content of cell surface HS as a result of transformation of mouse cells by either DNA (Papova-) or RNA (Leuko-) tumor viruses. Specifically, I have proposed that the cell surface HS forms a network with itself or other surface molecules, and that this network interacts with the cytoskeleton as well as with molecules on adjacent cells (intercellular communication). My current research is directed towards critical testing of these proposals through analyses of the interactions of cell surface heparan sulfate with membrane molecules as well as toward correlation of heparan sulfate structures with specific forms of cell social behavior.

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384. CONTROL OF NEOPLASTIC GROWTH

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OBJECTIVE: Role of animal glycosaminoglycans (GAGs) in adhesive and non-adhesive cellular interactions.

APPROACH: 1) Molecular: a) new methods for molecular characterization of GAGs: improved electrophoretic techniques, differential ethanol and polycation sensitivity, susceptibility to chemical and enzymatic degradative agents. b) Interactions with other matrix components (collagens, fibronectin) revealed by

affinity chromatography. 2) Cellular: involvement of GAGs in leukocyte contact activation, c) involvement of GAGs in leukocyte proliferation and peripheralization, d) role of GAGs in cellular sorting out, role of each GAGs class in cellular non-adhesive interactions with endothelial cell surfaces.

PROGRESS: 1) Separation of all known animal GAGs in a single electrophoretic run. 2) Evidence for a clear-cut GAGs shift of leukocyte surface following contact activation. 3) Evidence for a direct role of heparin sulfate in leukocyte peripheralization. C6S plays a basic role in regulating non-adhesive cellular interactions and is present in all the non-adhesive surfaces so far examined.

385. COMPLEX SACCHARIDES OF NORMAL AND CANCER CELL NUCLEI

Bhavanandan, V. P., Pennsylvania State University, Milton S. Hershey Medical Center, School of Medicine, Dept. of Biological Chemistry, *500 University Dr., Hershey, Pennsylvania*, 17033, United States of America

The objectives of this project are to study the nature of the complex saccharides (acid polysaccharides, glycoproteins, glycolipids) associated with the nuclei of normal and cancerous mammalian cells; their localization in the nucleus and their biological function with special emphasis on possible alterations in oncogenic cells. Normal and virus transformed (e.g., herpes simplex, SV40, cytomegalovirus, hamster sarcoma) fibroblasts, B16 mouse melanoma, human melanoma, rat liver cells and rat hepatoma cultured cells will be employed for this purpose. Solid tumors (e.g., melanoma, hepatoma) will also be utilized when preparative quantities of specific products are desired.

Nuclei will be isolated from cells labeled with (3H)glucosamine and ionized 35SO4 and the complex saccharides solubilized by treatment with proteolytic enzymes or with detergents. The acidic polysaccharides and glycoproteins will be separated from each other by precipitation with quaternary ammonium salts. After further fractionation by chromatography (gel filtration, ion exchange) the components will be characterized by chemical and enzymatic methods. Localization of these components in the nucleus will be attempted by examination of the nuclei and nuclear membrane by electron microscopy and autoradiography.

The biological functions of these polymers in the nuclei will be investigated by determining their qualitative and quantitative changes during the cell cycle, as a function of cell density and as a consequence of malignancy. In addition, the influence of exogenously added or removal of endogenous complex saccharides on the in vitro DNA and RNA synthesis and on the ion binding properties of the nuclei will also be determined. These studies constitute a new approach to understanding the uncontrolled growth of cancer cells by examining the role of endogenous nuclear mucopolysaccharides in the initiation and control of cell division.

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386. EARLY DETECTION OF TRANSFORMED CELLS

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Chromatographic and electrophoretic techniques will be used to analyze the surface mucopolysaccharide composition of cells infected with a temperature sensitive transforming virus. Immunofluorescence microscopy will be used to examine the distribution of hyaluronic acid and its association with other surface components. Alterations in type, relative amounts and distribution of mucopolysaccharides, during transformation, will be correlated with known properties of transformed cells e.g., decreased fluorescamine labeling, increased glucose uptake and loss of adhesion. A functional role for hyaluronic acid in these parameters will be investigated. We also propose to develop a flow cytometry assay to detect carcinogens, based on the differential labeling of normal and transformed cells with fluorescamine. This assay has potential application for the early detection of cancer cells.

387. BIOCHEMISTRY OF MUCOPOLYSACCHARIDES

Davidson, E. A., Miljkovic, M., Pennsylvania State University, Milton S. Hershey Medical Center, School of Medicine, Dept. of Biological Chemistry, *500 University Dr., Hershey, Pennsylvania*, 17033, United States of America

We intend to utilize stereospecific reaction paths for the synthesis of branched chain antimetabolites, to separate, purify, and chemically characterize the family of unusual anionic glycans synthesized by B16 melanoma cells, and a recently established human melanoma line, and to correlate, with various phases of the cell cycle, the production of these cell-associated matrix components in synchronized cells. In addition, sequencing of the peptide component of the cartilage proteoglycan will be continued.

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388. CANCER DETECTION BY SERUM ANALYSIS OF HETEROGLYCANS

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This proposal is for support of research to explore a biochemical method of cancer detection based on the quantitation of serum or plasma levels of a lipid-soluble sialic acid (LASA) fraction which is consistently and markedly elevated in tumor-bearing animal models and in human cancer patients. Specific objectives are: 1) To maximize reproducibility and sensitivity of the assay procedure; 2) to correlate LASA levels with tumorigenesis in a broad range of animal models and with several tumor types to gain information on the generality of the procedure as a diagnostic tool; 3) to isolate and identify chemically the chemical constituents of the LASA fraction which are elevated as a result of tumor burden in order to provide basic information necessary to understand the underlying biological mechanisms and to increase specificity and sensitivity of the assay procedure.

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389. ROLE OF GLYCOSAMINOGLYCANS IN MELANOMA METASTASES (MICE)

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This is part of a broader project. A summary of this subproject is not available.

390. BIOSYNTHESIS OF GLYCOSAMINOGLYCANS (RATS, CHICK EMBRYOS)

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This is part of a broader project. A summary of this subproject is not available.

B. OTHER ASPECTS OF CARBOHYDRATE METABOLISM IN NORMAL AND NEOPLASTIC CELLS

391. MOLECULAR STRUCTURE OF CHINESE HAMSTER OVARY CELL SURFACE CARBOHYDRATES - STRUCTURAL HETEROGENEITY OF HUMAN MYELOMA IGG CARBOHYDRATE

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OBJECTIVE: (a) To harvest, fractionate and identify in molecular detail the carbohydrates present on the surfaces of Chinese hamster ovary cells and mutants thereof selected for their resistance to the cytotoxicity of lectins (in particularly, wheat germ agglutinin). (b) To determine the extent of heterogeneity in carbohydrate structure for various human myeloma IgG's of the same subclass in order to explore the role of the primary sequence in the specification of the structural class of carbohydrate present.

APPROACH: Pronase digests are fractionated by a combination of high voltage electrophoresis, Biogel and lectin affinity chromatography. Structural identification is performed by 360 MHz proton magnetic resonance.

392. STUDIES ON THE ROLE OF CELL SURFACE CARBOHYDRATES IN THE DIFFERENTIATION OF DICTYOSTELIUM DISCOIDEUM

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OBJECTIVES: To study the role of cell surface carbohydrates in cell-cell interaction in Dictyostelium discoideum.

APPROACH: The changes in cell surface carbohydrates during the establishment of cell-cell contacts during the differentiation of Dictyostelium discoideum will be assessed by biochemical and immunological analyses of purified plasma membrane preparations. In addition, plasma membranes will be fractionated and the isolated components assessed for their ability to block 'in vitro' cell adhesion. Finally, attempts will be made to identify the molecules that induce spore and stalk cell differentiation.

393. INVESTIGATION OF THE DIFFERENCES IN CARBOHYDRATE STRUCTURES OF GLYCOCONJUGATES IN RELATION TO ABNORMAL CELL GROWTH

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OBJECTIVE: The aim of this project is the study of the primary structures of the carbohydrate chains of glycoconjugates derived from malignant cells in comparison to those from normal cells. The composition and structure of membrane glycoproteins from normal and malignant cells are of considerable interest because of their presumed role in growth control, intercellular adhesiveness and cell recognition. There are several indications that changes in the carbohydrate compositions of membrane glycoproteins are related to malignancy. Malignant cells bear relatively more bound carbohydrate groups of a greater molecular weight than corresponding control cells. It has been proposed that the carbohydrate structures from transformed cells are more complex (or higher branched) than those from normal cells. The first object of this study is to characterize the changes in terms of primary structures.

APPROACH: Two aspects can be distinguished: a) Isolation and purification of the carbohydrate chains; b) Structure determination of the carbohydrate chains by means of high resolution 1H-NMR spectroscopy in conjunction with methylation analysis. This approach was introduced by us in 1977 and successfully applied to the analysis of numerous N-and O-glycosidically linked carbohydrate chains of glycoproteins. J.F.G. Vliegenthart, H. van Halbeek and L. Dorland (1980) in IUPAC 27th Congr. Pure and Appl. Chemistry, Helsinki 1979 (Varmavuori, A., Ed.) pp. 253-262 Pergamon Press, Oxford. J.F.G. Vliegenthart, H. van Halbeek and L. Dorland (1981) Pure Appl. Chem. 53, 45-77.

PROGRESS: Progress has been made in the analysis of the carbohydrate chains of several glycoproteins which are considered to be markers of malignancy as well as in the analysis of glycopeptides derived from cell membranes of normal and transformed cells in cell cultures.

394. REGULATION OF GLYCOLYSIS IN YEAST

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Glycolysis is the major route of glucose catabolism in eukaryotic cells. In fact, the glycolytic enzymes constitute a large percentage of yeast cell total protein. The aim of the proposed research is to study the regulation of glycolysis at the level of enzyme synthesis in the simple eukaryote, Saccharomyces cerevisiae.

A yeast strain carrying a single-site nuclear gene mutation (gcr1) that results in an alteration of glycolytic enzyme levels has been isolated. The gcr1 mutation prevents the formation of the normal constitutive level of the glycolytic enzymes and appears to be involved in the control of the synthesis of this major class of proteins. The exact nature of the gcr1 defect is unknown.

The basis of this research proposal is the isolation of many mutants with the Gcr phenotype. The number of genes conferring this phenotype will be determined and the genes mapped. The function of the Gcr gene product(s) will be determined using a combination of media and temperature shift experiments in the presence of various inhibitors of macromolecular synthesis. Analysis of the results will determine if the block in glycolytic enzyme synthesis in the mutants occurs at the level of transcription, post-transcriptional modification, message stability, translation, post-translational modification, or enzyme stability.

395. SUGARS, GLYCOPROTEINS - NORMAL AND MALIGNANT CELLS

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Glucose transport in chick embryo fibroblasts is derepressed as cells replicate in a medium containing only traces of niacin or niacinamide. Measurement of NAD plus-NADP plus reveals that the total pyridine nucleotide is reduced to as little as 10% of normal after several cell divisions. An effort will be made to clarify the mechanism of transport carrier turnover which seems to be inhibited in the NAD plus depleted cells. The synthesis of NAD from niacin and adenosine will be studied as will the effect of analogues of NAD plus and niacin on the rate of carrier turnover in normal and in NAD plus-poor cells. Both transformed (malignant) and non-transformed cells will be studied. The effect of niacin and thiamine on the growth of P388 lymphosarcoma cells in suspension culture and in the peritoneum will be examined. In addition the influence of carbon source on the metabolic consequences for P388 tumor cells of vitamin depletion of the host CDF mouse is currently under study.

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396. CONTROL OF GLYCOLYSIS AND LACTIC ACID SECRETION IN CANCER CELLS BY BIOFLAVONOIDS AND OTHER COMPOUNDS

Racker, E., Cornell University, Ithaca Campus, School of Arts & Sciences, Dept. of Biochemistry Molecular & Cell Biology, *Ithaca, New York*, 14850, United States of America

We plan to analyze the inefficiency of the Na ion K ion pump ATPase from Ehrlich ascites tumor cells. The purified enzyme from the tumor will be compared with the enzyme from normal brain and kidney. Among the possibilities for the alteration of the enzyme, phosphorylation by protein kinase will be explored. Further analogs of lactate will be synthesized to block lactate excretion. Among them will be derivatives of phosphollactate. We plan to purify the lactate transporter and test it in

reconstituted vesicles. Effective inhibitors of lactate excretion will be tested in tissue culture at various temperatures in order to explore the effect of intracellular pH on killing by hyperthermia.

397. GLUCOSE-INSULIN KINETICS - MODELING OF CARBOHYDRATE METABOLISM

Berman, M., Foster, D., Hetenyi, G., Hall, S. E., McGuire, E. A., Roth, J., Brennan, M., Burke, M., Watsney, M., Riemke, R., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Mathematical Biology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Kinetic models of the glucose regulatory systems are being developed for man and other animals in collaboration with several experimental groups. These include models of the distribution, metabolism and receptor binding of insulin, using data from both radioiodinated and native insulins, and the production and utilization of glucose as elucidated by tracer studies with labeled glucose, alanine and lactate. Models of the role of insulin in the control of glucose utilization and production are also being studied.

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398. GLUCOSE-CONTAINING TETRASACCHARIDE IN HUMAN URINE

Zopf, D. A., Levinson, R. E., Pizzo, P. A., Lundblad, A., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Ultrastructural Pathology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

A glucose-containing tetrasaccharide with the chemical structure Glc alpha 1-6Glc alpha 1-4Glc alpha 1-4Glc is excreted in abnormally large amounts in the urine of individuals with glycogenoses and in some types of muscular dystrophy (G. Lennartson et al. Biomed. Mass Spectrometry 3: 51-54, 1976). This oligosaccharide probably represents an abnormal metabolite of glycogen in that it occurs as a limit dextrin after exhaustive treatment of glycogen with amylase. Using an immunogen prepared by coupling the oligosaccharide to the protein carrier KLH we have obtained specific antibodies that can be used to detect 100 picomoles of the reduced oligosaccharide by radioimmunoassay. This assay will be used to study the relationship between glycogen metabolism and excretion of the oligosaccharide.

399. GLYCOSAMINOGLYCANS IN NORMAL AND MALIGNANT CELLS

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Glycosaminoglycans (GAG) are polysaccharides abundant in extracellular matrix and on cell surfaces. No precise biological role has been assigned to these substances, but they are thought to be involved in the maintenance of the integrity of the connective tissue.

We have recently found that a subfraction of heparin and heparan sulfate enhance the binding of fibronectin to collagen by becoming associated with the fibronectin-collagen complex. This suggests that the presence of all three main components of extracellular matrix, collagen, fibronectin and GAG, may be necessary for the formation of the matrix. The disturbed deposition of extracellular matrix is often seen in transformed cells could be due to disturbances in the subfraction of GAG that enhance the association between fibronectin and collagen. We propose to isolate by affinity chromatography on fibronectin-collagen complexes the fractions of heparin and heparan sulfate with this activity and study their effect on the formation of extracellular matrix by normal and transformed cells. We will also determine whether cultured fibroblasts produce GAGs (or proteoglycans) which bind to fibronectin-collagen complexes and isolate them. Preliminary results suggest that such substances are found in spent culture media. We will study their effects on the growth characteristics of cells and determine whether differences in their expression or characteristics show variations that correlate with cellular parameters such as growth, transformation and malignancy.

These studies may contribute to the understanding of the role GAGs play in the organization of extracellular matrix, the attachment of cells to this matrix, and how these functions are disturbed in malignant cells.

BIBLIOGRAPHIC REFERENCES: Shigeo Sakashita, Eva Engvall and Erkki Ruoslahti (1980) Basement Membrane Glycoprotein Laminin Binds to Heparin, FEBS Letters 116:243-246.

400. ROLE OF GLYCOSAMINOGLYCANS AND BINDING PROTEINS IN CONTROL OF TUMOR CELL ADHESIVE, MOTILE AND INVASIVE BEHAVIOR (ABBREV)

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OBJECTIVE: The intent of this project is to determine the molecular mechanisms of invasion, specifically the role of glycosaminoglycans and binding proteins in regulating cell adhesion and motility, and therefore, invasive properties during metastatic infiltration.

APPROACH: We are examining the glycosaminoglycan synthesis at the sites of metastasis, correlating adhesive behavior on glycosaminoglycan substrata in vitro with metastatic capabilities in vivo; investigating the role of cell surface hyaluronic acid in determining adhesive/motile behavior in vitro; characterizing glycosaminoglycan binding proteins, the connectins, their binding properties, their presence in tumor and normal tissues, and their effect on tumor cell adhesion and motility; and assessing the role of surface glycosyltransferases in tumor cell movement.

PROGRESS: To date, most information available on the involvement of glycosaminoglycans and proteins in cell movement originates from studies of embryonic tissues. Several studies suggest that neural crest is actively motile on only sulphated substrata, that they require cell surface hyaluronic acid for this behavior, and that affinity purified connectins of MW 67, 60, and 42 K bind to glycosaminoglycans, increase cell-sulphated substratum adhesion, promote cell-cell aggregation, and inhibit cell movement. Given the similarities between motile embryonic cells and metastatic tumor cells, it is predicted that both glycosaminoglycans and connectins will influence tumor cell adhesive/motile properties.

401. FUNCTIONAL ROLE OF OLIGOSACCHARIDES IN CANCER

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OBJECTIVE: To identify and determine the functional role (if any) of oligosaccharides in the pathogenesis of cancer.

APPROACH: Oligosaccharide determinants on glycoproteins, glycolipids and glycosphingolipids are identified. Promising oligosaccharide sequences are synthesized chemically with their analogs. Monoclonal antibodies are produced and their specificity to the synthetic and natural oligosaccharide sequences will be examined. Through the use of mouse models, the effects of antibody administration will be examined with regards to tumor progression, metastasis and expression of tumor-specific or tumor-associated oligosaccharides.

402. STUDIES OF MAMMALIAN GLYCOPROTEIN OLIGOSACCHARIDES

Gottlieb, C. C., Washington University, School of Medicine, Dept. of Anatomy & Neurobiology, *660 S. Euclid Ave., St. Louis, Missouri*, 63110, United States of America

Studies are being performed to obtain an estimate of the structural variety of complex carbohydrates synthesized by a single mammalian cell type in culture. Variants of this cell line with alterations in membrane carbohydrates due to in vivo defective glycosyltransferase activities are examined for involvement of the same defects in biosynthesis of carbohydrates on non-membrane glycoproteins.

BIBLIOGRAPHIC REFERENCES: Gottlieb, C., Kornfeld, S., and Schlesinger, S. 1979 Restricted replication of two

alpha viruses in ricin-resistant mouse L-cells with altered glycosyltransferase activities. J. Virol., 29: 344-351.

403. OLIGOSACCHARIDE STRUCTURE AND FUNCTION IN RECOGNITION

Baenziger, J. U., Washington University, School of Medicine, Dept. of Pathology, *660 S. Euclid Ave., St. Louis, Missouri*, 63110, United States of America

The objective of this proposal is to examine the role of glycoprotein oligosaccharides as information-bearing macromolecules involved in inter- and intracellular communication. This project has three phases, the first of which is to define the structure of the oligosaccharides present on the high uptake form of human Beta-glucuronidase, human Beta-hexosaminidases A and B, an IgD myeloma protein, the galactose-specific carbohydrate binding protein (Gal-CBP) of human hepatocytes, and other vertebrate carbohydrate-binding proteins (CBPs) detected by affinity labeling. A method for microsequencing utilizing the introduction of fluorescent probes onto oligosaccharides and separation by high performance liquid chromatography is presented for use in such studies. Altered exposure of oligosaccharides following ligand binding by monoclonal IgG and IgM, and haptoglobin will also be examined as a potential source of specificity. The second phase will consist of the synthesis of photoactivatable, 125I-labeled oligosaccharide affinity probes and their utilization to identify, isolate, and examine vertebrate CBPs. The third phase will involve detailed studies of the CBPs thus identified and isolated. The specificity of CBPs will be defined by carrying out binding studies with glycoproteins and oligosaccharides of known structure. The behavior of the CBP in intact cells will be examined using the glycoproteins and oligosaccharides as well as by affinity labeling which will permit direct examination of the CBP in the cell. CBPs will be incorporated into liposomes to examine their potential role in cell-cell recognition and to introduce purified CBP into other cells such as fibroblasts by fusion. Introduction of the receptor into cells will make it possible to determine the components involved in endocytosis by CBP.

BIBLIOGRAPHIC REFERENCES: Baenziger, J.U. and Natowicz, M. (1981) Rapid Separation of Anionic Oligosaccharide Species by High Performance Liquid Chromatography. Anal. Biochem. 112, in press. Mellis, S.J. and Baenziger, J.U. (1981) Separation of Neutral Oligosaccharides by High Performance Liquid Chromatography. Anal. Biochem. 112, in press.

404. A COMPARISON OF OLIGOSACCHARIDE OF SINDBIS VIRIS AND VSV

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This is part of a broader project. A summary of this subproject is not available.

405. A POSSIBLE ROLE OF BETA-ENDORPHIN IN CARBOHYDRATE METABOLISM IN RAT MAMMARY GLANDS

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It is becoming more and more clear that newly discovered opiate-like peptides in general, and beta-endorphin in particular, affect the endocrine environment in a significant manner. Both adrenocorticotropic hormone (ACTH) and beta-endorphin are synthesized from a common precursor and since ACTH, through its action on the regulation of hydrocortisone synthesis and secretion affects the generation of energy in mammary glands, we decided to investigate whether beta-endorphin had a role to play in the same process. Naloxone hydrochloride is a specific antagonist of the action of opiate-like peptides and therefore we decided to try to elucidate the role of beta-endorphin by studying the effects of the administration of Naloxone on carbohydrate metabolism in rat mammary glands and comparing them with those of hydrocortisone. Naloxone itself has no effect on the activities of any of the enzymes studied in the mammary glands from hypophysectomized animals. Therefore, it appears that the changes observed in enzyme activities may be the result of the blocking of beta-endorphin action on mammary glands.

Naloxone treatment stimulated the activities of phosphofructokinase, glucose-6-phosphate dehydrogenase and alpha-glycerolphosphate dehydrogenase. Similarly, the administration of hydrocortisone also stimulated the activities of the same enzymes. However, since Naloxone stimulation indicates a possible inhibition of these enzyme activities by beta-endorphin, it appears that ACTH and beta-endorphin play opposing roles in carbohydrate metabolism in rat mammary glands. Further work is in progress to elucidate this mechanism.

406. THE REGULATION OF CARBOHYDRATE METABOLISM IN ANIMAL CELLS IN CULTURE

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OBJECTIVE: The principal purpose of this investigation is to study the control of carbohydrate metabolism in animal cells by isolating mutants of Chinese hamster ovary cells.

APPROACH: Suitable, well characterized mutants will be subjected to detailed investigation to obtain information on the regulation of gene expression, the regulation of enzyme activity by molecular effectors and the organization of the genes on hamster and human chromosomes. The results of these studies should have application in strategies designed to modify the consequences of derangements of such controls in neoplasia and inherited disease.

PROGRESS: Chinese hamster ovary cells will grow on only a few energy sources besides glucose. They will not grow, for example, on lactose, maltose, ribose or lactate. We have isolated mutants which will grow on additional energy sources and are determining the mechanism by which these new phenotypes arise. Our present data indicate that previously unexpressed genes are being expressed in some of the mutants. Should this be confirmed we will be in a position to study the state of the particular gene in the parental (non-expressing) strain and in the mutant (expressing) strain.

407. EFFECTS OF ANDROGENS ON CARBOHYDRATE METABOLISM IN RAT MAMMARY GLANDS

Deshpande, N., Mitchell, I., Imperial Cancer Research Fund, Laboratory for Metabolic Studies in Cancer, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

The role of androgens in the normal development and function of the mammary gland is unclear. Most published workers have found an inhibitory role for the hormone in the growth of the gland. Therefore we decided to investigate whether the mechanism of action of the hormone on the mammary gland is mediated via inhibition of glycolysis.

Intact and ovariectomized adrenalectomized rats were treated with testosterone propionate either alone or in conjunction with cyproterone acetate (an inhibitor of androgen action) for 10 days. The mammary glands were removed when the animals were killed and the activities of some of the key enzymes of carbohydrate metabolism were measured. Administration of the androgen stimulated the activities of phosphofructokinase, glucose-6-phosphate dehydrogenase, 6-phosphogluconate dehydrogenase and lactate dehydrogenase in the mammary glands of these animals and cyproterone acetate inhibited these testosterone-induced increases in activity. Thus our results indicate that androgens probably play a regulatory rather than an inhibitory role in mammary gland growth.

Further experiments are in progress to elucidate these findings.

408. EFFECT OF GLUCOCORTICOID TREATMENT ON CARBOHYDRATE METABOLISM IN HUMAN BREAST CARCINOMAS

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Recent studies have shown that glucocorticoids play an important role in the regulation of growth of human breast carcinomas. About 10 percent of patients with metastatic disease respond to glucocorticoid treatment and the same treatment in patients with early disease results in a considerable prolongation of the disease-free interval. Studies on cell cultures of human carcinomas indicate that cell growth can be retarded by the

presence of glucocorticoids in the medium. Our own studies on the effect of hydrocortisone on carbohydrate metabolism in rat mammary glands indicate that the hormone regulates the activity of alpha-glycerolphosphate dehydrogenase (alpha-GPDH), an important enzyme in the transfer of substrates into lipid deposition, thus reducing the concentrations of substrates available for energy generation and growth. Therefore it was decided to investigate whether a similar phenomenon could be the cause of alterations in the growth rate of human carcinomas. This investigation is carried out on patients with early disease who are undergoing mastectomy.

These patients are treated with prednisolone (3 x 5 mg/day for 3 days) prior to mastectomy and at operation the carcinoma is excised and retained for biochemical investigation. This work has been in progress for the past eight months.

409. REGULATION OF CARBOHYDRATE METABOLISM

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We will examine channeling of intermediates between glyceraldehyde-P dehydrogenase and phosphoglycerate kinase in solution and on the red cell membranes. We are interested in the origin and toxicity of methylglyoxal. How much is a consequence of the side reactions of aldolase and triose-P isomerase?

BIBLIOGRAPHIC REFERENCES: Lithium Action on Glucose Uptake in Brain; Role of Glucose-1,6-P2 as a Regulator of Hexokinase. Irwin A. Rose and Jessie V. B. Warms. Biochem. Biophys. Res. Commun. 92:1030-1036, 1980. Glucose Exchange and Catalysis by Two Crystalline Hexokinase. Glucose Complexes: Evidence for an Obligatory ATP-Dependent Conformational Change in Catalysis. Keith D. Wilkinson and Irwin A. Rose. J. Biol. Chem. 255:7569-7574, 1980.

410. GALACTOSYLTRANSFERASE AND LACTALBUMIN OF THE LACTOSE SYNTHETASE SYSTEM

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The major thrust of this work is to learn the structural and mechanistic properties of a galactosyltransferase which, in conjunction with alpha-lactalbumin, is involved in the biosynthesis of lactose. Alpha-lactalbumin isolated from the milk of most species does not contain carbohydrate. In contrast, alpha-lactalbumin isolated from rat milk is fully glycosylated and the question is why this is the case. Also, there is considerable interest in the mechanism of glycosylation of proteins and in particular the involvement of lipid intermediates in the transfer of carbohydrates to an asparagine residue. Rat alpha-lactalbumin is an excellent model system for such studies since it is easy to isolate and can be synthesized in slices, tissue pieces and certain mammary tumors. In addition, its synthesis is under hormonal control. For such studies to be meaningful it is necessary that the amino acid sequence of rat alpha-lactalbumin and the sequence of the carbohydrate units be completed. Progress in both of these areas has been made. The amino acid and carbohydrate sequence of rat alpha-lactalbumin will be completed. Studies on galactosyltransferase with respect to the carbohydrate units and chemistry of the active site will be continued.

411. GROWTH OF VERTEBRATE CELLS WITHOUT EXOGENOUS SUGAR

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Our earlier studies showed that glutamine oxidation can provide most or all cell energy in a variety of cultured vertebrate cells. 1) We intend to extend these analyses to specific tissues to determine to what extent glutamine is a universal energy source. Metabolism of glutamine and sugar will be monitored. 2) We have grown cultured cells in a sugar-free media supplemented with a pyrimidine riboside. We hope to identify the early reactions of uridine metabolism and its regulation to understand why most ribosides do not support growth. 3) Glucose metabolism depresses glutamine utilization 3 fold. We shall use specific mutants to determine the reactions of hexose metabolism responsible. 4) Conversely, little hexose carbon entered the citrate cycle in cultured cells and we shall try to culture cells in glutamine-free media to determine if its metabolism is responsible. 5) We observed a Pasteur Effect in HeLa cells and shall test for a regulated reaction late in glycolysis by work with mutants and measurements of internal levels of key intermediates.

412. SYNTHETIC CARBOHYDRATE-PROTEIN CONJUGATES

Marshall, J. J., Pollard, M., Mizokami, K., University of Notre Dame, College of Science, Dept. of Microbiology, *Notre Dame, Indiana,* 46556, United States of America

We will continue studies on the attachment of carbohydrates to enzymes and nonenzymic proteins to give soluble carbohydrate-protein conjugates. We will compare, in vitro, properties of native and conjugated enzymes, in particular the effect of different carbohydrates and different coupling procedures on the stability properties and other characteristics of the conjugates. The behavior of conjugated proteins and enzymes in experimental animals will be investigated, with particular regard to defining the characteristics of the conjugates that determine their behavior in vivo, and we will evaluate their possible applications as therapeutic agents, for example in the treatment of inborn errors of metabolism and other metabolic disorders. Thus we will investigate in detail the effect of different carbohydrates on the immunological properties and circulatory lifetimes of conjugated proteins, and study the 'homing' of conjugates to different organs and tissues. Experimental enzyme therapy will be conducted with conjugated enzymes, using experimental animals and cultured cells. We will prepare carbohydrate derivatives of L-asparaginase and test their effectiveness as anti-tumor agents in animals bearing implanted lymphosarcomas; it is hoped that the usefulness of this enzyme as an anti-tumor agent can be improved by coupling with carbohydrate, by virtue of extension of the circularity lifetime of the enzyme and elimination of the allergic reactions associated with its use. The properties of cyclodextrin-albumin as a drug carrier will be evaluated. Attempts will be made to develop blood substitutes by conjugation of hemoglobin with polysaccharides such as hydroxyethyl starch and dextran.

BIBLIOGRAPHIC REFERENCES: Preservation of Enzymes by Covalent Attachment to Dextran, J. John Marshall, A.C.S. Symposium Series (1980) 123, 125-143. Preparation and Characterization of Dextran-Glycerol Kinase Conjugates J.J. Marshall, P.F. Lue and C.V. Flaherty, in press (1981).

413. COMPLEX CARBOHYDRATE RELEASED FROM MAMMALIAN CELLS BY TRIFLUOROACETOLYSIS

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Trifluoroacetolysis is a recently-developed method that releases oligosaccharides intact from glycoproteins and glycolipids. Carbohydrate chains released as a mixture from whole tissues, tissue fractions, or cells grown in culture, are easily recovered in nearly quantitative yield and reconstituted to their native form. Analysis of the majority of oligosaccharides containing six or fewer monosaccharide units is performed by combined gas chromatography and mass spectrometry of permethylated, N-trifluoroacetylated oligosaccharide derivatives. Analysis for certain specific oligosaccharides is carried out by radioimmunoassay using antibodies produced against purified oligosaccharides coupled to polypeptide carriers. It is anticipated that the repertoire of oligosaccharide chains produced by cells or tissues will reflect states of cellular differentiation and reveal potential cell surface markers.

414. FETAL LIVER AND HEPATOMA IN ORGAN CULTURE

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The purpose of this project is to study the metabolism of carbohydrates in explants of fetal rat liver hepatomas maintained in organ culture. We will study the effects of corticosteroid, glucagon, insulin, and cyclic nucleotides on the development of gluconeogenesis in these tissues, in order to evaluate if the known structural similarities between fetal liver and hepatoma are reflected in functional similarities as well.

Techniques will be developed for growing tumors under organ culture conditions. Carbohydrate metabolisms of growing and static tissues will be compared.

BIBLIOGRAPHIC REFERENCES: Changes In The Properties of Fetal Rat Liver During Organ Culture, Carl Monder and Alena Hatle Coufalik, In Vitro 15, No. 8, 579-586 (1979).

415. SYNTHESIS OF BIOLOGICALLY IMPORTANT CARBOHYDRATES

Horton, D., Priebe, W., Takagi, Y., Mols, O., Varela, O., Bhate, P., Chang, Y., Czuchra, J., Larson, G., Warner, S., Ohio State University, Columbus Campus, School of Mathematics & Physical Sciences, Dept. of Chemistry, *164 W. 17th Ave., Columbus, Ohio*, 43210, United States of America

The program is concerned with development of reactions and procedures in the carbohydrate field useful for synthesis of structures present in biologically important carbohydrate derivatives, and in understanding their biological role. A general objective is the establishment of reactions of high specificity that can be used, under mild conditions, for the structural conversion of common sugars and their derivatives into analogs of unusual functionality or stereochemistry that are present in numerous classes of natural carbohydrate derivatives, and also other chiral molecules, having importance in essential biological function and metabolism, or in therapeutic control. The reactions and procedures will facilitate partial and total syntheses of such unusual sugars as are present in various antibiotics, components of bacterial cell walls, antitumor agents, physiologically active glycosides, and sugar derivatives involved in metabolism; analogs of other biologically active molecules involved in neurotransmission and other functions will be obtained optically pure by carbohydrate-based syntheses.

BIBLIOGRAPHIC REFERENCES: Elisabeth Fanton, Jacques Gelas, and Derek Horton, Novel Modes for Selective Protection of Ketose Sugars and Oligosaccharides of Biological and Industrial Importance, J. C. S. Chem. Commun., 21-22 (1980). Derek Horton and Robert A. Markovs, Carbon-chain Extension Through C-1 of 2-Deoxyaldose dithioacetal derivatives: A Route to 1,3-Dideoxy-2-ketoses, Carbohydr. Res., 78, 295-303 (1980).

416. YEAST - A MODEL FOR STUDYING REGULATION IN CANCER CELLS

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The long range goal of the proposed research project is to obtain an understanding of the mechanism of regulation in eukaryotic cells and to relate such information to the metabolism of cancer cells. The yeast Saccharomyces will be used as a model system. Cancer cells are characterized by an altered growth rate and morphology (both of which can be reversed by cyclic AMP in certain cultured tissues) and an altered respiration. Therefore, more specifically, glucose repression and its role in the regulation of enzyme synthesis and mitochondrial biogenesis will be studied by isolating mutations involving these aspects of yeast metabolism.

In yeast, the biogenesis of mitochondria and the synthesis of the maltose, galactose and melibiose fermentation enzymes is repressed by glucose. Methods are described by which one can isolate mutants of the glucose repression system. The mutants will be characterized genetically to determine the mode of inheritance (cytoplasmic or nuclear, number of loci, etc.), and biochemically by enzyme determinations for a number of glucose sensitive loci and cyclic AMP levels. (This should indicate whether cyclic AMP is of central importance for glucose repression in yeast, as it is in bacteria.)

The effect of glucose on the transcription of glucose repression sensitive loci in varying states of repression and derepression in both wild-type and mutant strains will be determined. These studies will be done by DNA-RNA hybridization in which the DNA probe will be a recombinant plasmid carrying the glucose sensitive yeast gene.

417. METABOLIC STUDY OF ADULT AND PEDIATRIC CANCER PATIENTS

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The overall objective of this research is to investigate glucose and lactate metabolism in hospitalized patients with malignant solid tumors, particularly colo-rectal cancer. Using isotope tracer infusion techniques, the production, oxidation and recycling of glucose via lactate will be measured under standardized conditions. These data will be correlated with the presence or absence of carbohydrate intolerance following an oral glucose load. Preliminary data on the metabolic response of cachetic patients to an ingested protein meal will be extended and incorporated into planned work with alanine turnover studies. These data on abnormal responses to ingested protein may provide a rational basis from which improvements, concurrent therapy and support may be achieved.

BIBLIOGRAPHIC REFERENCES: Lactate metabolism in patients with metastatic colo-rectal cancer. Holroyde, C.P., Axelrod, R.S., Skutches, C.L., Haff, A.C., Paul, P., and Reichard, G.A. Cancer Research 39:4900-4904, 1979. Carbohydrate metabolism in cancer cachexia. Holroyde, C.P. and Reichard, G.A. Accepted for publication. Cancer Treatment Reports. 1981.

418. SINGLE AND CO-CARBON SOURCE UTILIZATION AND MAMMALIAN CELL METABOLISM

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In recent years evidence has accumulated that mammalian cells can grow on sugars other than glucose. Moreover, growth on alternative carbon sources results in phenotypic changes involving cell morphology, sugar transport, lectin binding and the specific glycoproteins associated with the cell membrane.

Recent work in the laboratory has produced evidence for variations in hexokinase and other sugar kinase activities as a function of the carbon source on which cells have grown. These effects are not identical in normal and transformed cells. Surprisingly little of sugars other than mannose and glucose is consumed by cells per cell replication even when growth of the culture is excellent. The supplementing of one sugar by another produces both inhibitory and stimulatory effects depending upon the sugars used and the cells in question. The intent is to study the mechanism of enzyme (e.g., hexokinase) regulation and to identify intermediates responsible for aberrant growth and glycolysis when combinations of sugars are used.

A further objective is the careful analysis of changes in sugar composition of a specific epiglycan (a surface glycoprotein of the mouse tumor cell TA3) occurring when the cells are cultivated on carbon sources other than glucose. The aim is to determine whether marked changes in the proportions of specific sugars in the oligosaccharide of a single glycoprotein mimic those reported for the total membrane carbohydrates. The implications for the modification of specificity of tumor antigens resulting from changes in sugar composition are of considerable potential interest.

419. BIOLOGY OF COMPLEX CARBOHYDRATES

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Oligosaccharides will be isolated from natural sources. Oligosaccharides will be coupled to macromolecules in order to make antibodies specific for certain sugar sequences. The antibodies will be used in radioimmune assays for the occurrence of these sugar sequences in the glycolipids and glycoproteins of cell membranes and serum in order to investigate the involvement of the complex carbohydrates of cell surfaces in development and regulation or as tumor-specific antigens.

Waldenstrom macroglobulins and myeloma proteins will be screened for their ability to react with human erythrocytes.

Those that do will be tested for their ability to react with various oligosaccharides coupled to macromolecules.

Carbohydrates on cell membranes that are receptors for toxins, hormones, and antibodies will be isolated and characterized.

BIBLIOGRAPHIC REFERENCES: Rohr, T.E., Smith, D.F., Zopf, D.A., and Ginsburg, V.: Leb-Active glycolipid in human plasma: measurement by radioimmunoassay. Arch. Biochem. Biophys. 199: 265-269, 1980. Smith, D.F., and Ginsburg, V.: Antibodies against sialyloligosaccharides coupled to protein. J. Biol. Chem. 255: 55-59, 1980.

420. REGULATION OF SUGAR METABOLISM IN TISSUE CULTURE CELLS (MAMMALS, BACTERIA)

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This is part of a broader project. A summary of this subproject is not available.

421. RADIOLABELLED INHIBITORS OF GLUCOGENESIS

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This is part of a broader project. A summary of this subproject is not available.

VI. STUDIES ON THE BIOCHEMISTRY OF LIPIDS OF NORMAL AND NEOPLASTIC CELLS

A. BIOSYNTHESIS AND METABOLISM OF STEROLS

422. CHOLESTEROL METABOLISM IN NORMAL AND MALIGNANT LIVER

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This laboratory has had a long-standing interest in the relationship between cholesterol synthesis and cancer. Three of our previous findings form the basis of this proposal: 1) that the synthesis of mevalonate is the primary site of cholesterol feedback regulation; 2) that the feedback control of mevalonate synthesis is consistently deranged in malignant and premalignant cells; and 3) that mevalonate, perhaps through the mediation of isopentenyl adenine, plays an essential role in DNA replication in normal cells. These observations will be extended by determining, in tissue culture and in vivo, whether mevalonate and isopentenyl adenine also control DNA replication in malignant cells. We shall also determine whether various chemical analogues of the HMG CoA reductase inhibitor, compactin, more effectively inhibit both mevalonate and DNA synthesis in normal and malignant cells in tissue culture and in vivo, and whether such inhibitors of mevalonate synthesis might cause retardation or regression of tumor growth. The biosynthesis of isopentenyl adenine both in normal and malignant tissue will be studied, as well as the role of isopentenyl adenine and chemically synthesized analogues in cell growth in vitro and in vivo. Finally, to elucidate the detailed enzymatic mechanism by which isoprene adenine stimulates DNA replication, we plan to determine the effect of compactin, mevalonate, and isopentenyl adenine on the specific classes of DNA polymerase as well as on DNA nicking-closing enzyme and unwinding protein.

SIGNIFICANCE: Our studies have demonstrated for the first time that mevalonate, independent of its function as a cholesterol precursor, is required for DNA replication. This finding, coupled with our previous observation that uncontrolled synthesis of isoprene precursors is a consistent characteristic of all malignant cells, suggests a mechanism by which this derangement on the pathway of cholesterogenesis may lead to abnormal rates of DNA synthesis and perhaps to malignancy. Finally, compounds that alter the rate of isopentenyl purine synthesis may offer a new approach to controlling abnormal cell replication.

BIBLIOGRAPHIC REFERENCES: Feingold, K.R., M.H. Wiley, G.L. Searle, B.K. Machida; M.D. Siperstein. Sex difference in human mevalonate metabolism. J. Clin. Invest. 66:361-366, 1980. Feingold, K.R., M.H. Wiley, G. MacRae, M.D. Siperstein. Mevalonate metabolism in pregnant rats. Metabolism, 1980 (in press).

423. MEMBRANES IN NORMAL AND CANCER CELLS

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OBJECTIVE: Investigations will be continued on the membranes of normal and transformed cells.

APPROACH: The biosynthesis of cholesterol, an important membrane component, will be studied in two Morris hepatomas, with particular attention to the characterization of their microsomal methyl sterol demethylase. Further studies on the role of the Na K ATPase in the high aerobic glycolysis of tumor cells will be carried out to verify that it is not an increased pumping of monovalent cations, but an inefficient operation (leaky pump) which is responsible for the defect. A group of natural plant products (bioflavonoids) were shown to repair the leaky pump. Search for an effective compound that could be tested in vivo is being continued. An additional line of inquiry concerns the role of proteolipids in transport and experiments are being directed towards testing the claim that cancer cells excrete proteolipids and towards determining whether changes in proteolipid patterns may be responsible for the defect in the Na K pump in tumor cells.

PROGRESS: This project covers the research activities of several investigators and progress was realized on the following topics. The studies on the permeability changes induced in transformed fibroblasts by external ATP were further characterized. The isolation and reconstitution of the glucose transporter was carried out. New methods were developed to measure 'membrane fluidity', or more specifically the lateral mobility of proteins and lipids. Whereas fluidity as measured by a lipid probe is the same in normal and transformed cells, the motion of specific proteins (e.g. membrane receptors labelled with Fab antibodies) appears to be reduced in transformed cells compared to controls. Changes in fluidity of membranes exposed to sodium linoleate have also been observed. Techniques for measuring ion fluxes have been developed and used to evaluate the response of cells to acetyl choline. Isolation of electrolectin as a homogeneous protein of 27,000 mol wt has been achieved and its location on the cell surface identified my making use of an antibody against electrolectin. Further studies have been carried out to elucidate the mechanism of lactate transport in erythrocytes and Ehrlich ascites tumor cells, including development of 'suicide inhibitors' that should allow for intracellular accumulation of lactic acid.

424. LIPID METABOLISM IN TUMORS

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Our previous studies have shown that mitochondria from various Morris hepatoma lines have increased content of phospholipid relative to protein as compared with that found in normal or regenerating rat liver (Cancer Res., 39: 2978, 1979). This is not an artifact due to a decreased mitochondiral protein content, and does not appear to be due to decreased phospholipase A activity in hepatoma mitochondria (J.Lipid Res., 19: 553, 1978). Several enzymes involved in the de novo synthesis of phospholipids have been found to be present in substantial quantities in hepatoma mitochondria, including CTP: phosphatidate cytidylyltransferase and the CA2 ion-dependent base exchange pathway for the synthesis of phosphatidylserine. The latter enzyme is absent from normal liver mitochondria (J.Lipid Res., 20: 607, 1979).

Current studies are focused on the cholesterol content of Morris hepatoma membranes. Our preliminary studies show that cholesterol, which is nearly absent from normal liver mitochondira, is present in substantial quantities in hepatoma mitochon-

dria. The fluidity of mitochondiral membranes is decreased as measured by fluorescent polarization spectroscopy. In vivo studies on the intracellular transfer of newly-synthesized cholesterol are planned. Collaborative studies with the group of Dr. K.W.A. Wirtz in the Netherlands have shown the near absence of the exchange protein which catalyzes the exchange of phosphatidylethanolamine (Biochim. Biophys. Acta, 600: 376, 1980). Interestingly, this protein, usually referred to as the universal phospholipid exchange protein, also catalyzes cholesterol exchange between membranes. The significance of these findings is being investigated.

425. CHOLESTEROL METABOLISM IN HEPATIC NEOPLASMS

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The ultrastructure and adaptation of altered hepatocytes, from the presumed premalignant nodules, induced by feeding acetylaminofluorene to rats, will be studied in monolayer culture after dissociating the liver cells by collagenase perfusion. The new cell populations will be identified by the presence of gamma-glutamyl transpeptidase and they will be further subdivided into less and more autonomous variants on the basis of their autoradiographic nuclear labelling index after in vivo injection of H-TdR. Their length of survival and resistance to AAF will also be determined. Selected aspects of cholesterol and bile acid synthesis in the induced lesions will also be studied in vitro and in vivo by assaying the levels of these pathways' respective rate controlling enzymes, HMG-CoA reductase and cholesterol 7alpha-hydroxylase and by determining the level of cholesterogenesis, reflected by in vivo incorporation of 3H2O into cholesterol-H. The perturbing of bile acid and cholesterol synthesis through dietary, hormonal and drug treatments should alter the activities of the enzymes in distinctive ways in normal liver. These alterations may then also be studied in preneoplastic and neoplastic liver to determine whether a change from the norm is due to tumor progression or if it represents a more basic effect of hepatic carcinogenesis.

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426. CHOLESTEROL IN MEMBRANE FUNCTION AND CELL TRANSFORMATION

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Substantial evidences have shown that cholesterol synthesis is an important metabolic process which is intimately related to the control of cellular proliferation and expression of several membrane associated functions. However the mechanism through which cholesterol synthesis is regulated is not fully understood. It is generally accepted that the rate-limiting step in the sterol synthetic pathway is the generation of mevalonate, an intermediate metabolite catalized by 3-hydroxy-3-methyglutaryl coenzyme A (HMG-CoA) reductase. We reason, therefore, that investigation of the mechanism by which the activity of HMG-CoA reductase is regulated is necessary to allow us to understand not only the control of sterol synthesis but also the regulation of cell growth and proliferation. Several oxygenated derivatives of cholesterol, e.g., 25-hydroxycholesterol, specifically inhibit the activity of HMG-CoA reductase either by repression of the enzyme synthesis or by acceleration of the enzyme degradation. Several mevalonate-like fungal metabolites, e.g., compactin, competitively inhibit the activity of HMG-CoA reductase when they were incubated with cell cultures. Upon removal of these metabolites by washing the level of HMG-CoA reductase was found to increase by a still unknown mechanism. The objective of the present research project is to investigate the mechanisms by which oxygenated sterols, mevalonate-like fungal metabolites and other metabolic inhibitors affect the activity of HMG-CoA reductase.

427. STEROLS AND THE BIOGENESIS AND FUNCTION OF SURFACE MEMBRANES

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As a group of macromolecular structures containing largely lipid and protein, biological membranes are characterized by great diversity with respect to their specific components. This variety undoubtedly is related to structural and functional differences between the membranes. Among the lipid components of eukaryotic cultured animal cells, this project is concerned with the isolation and characterization of mutants which have lower plasma membrane sterol contents, with the interactions between membrane lipids, and with surface membrane lipid metabolism.

428. EFFECTORS OF CHOLESTEROL SYNTHESIS

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I propose to determine, by direct methods, the regulatory mechanism(s) by which hepatoma tissue culture (HTC) cells control their level of HMG CoA reductase (HMGR) in response to low density lipoproteins, cholesteryl succinate, and 25-hydroxycholesterol. To accomplish this broad goal, I plan to a) isolate and purify antibodies to normal Buffalo rat liver HMGR, b) determine with anti-HMGR whether the increased catalytic activity of HTC cells maintained in medium which contained lipoprotein poor serum is, in fact, the result of an enhanced rate of HMGR synthesis (Ks) or of a decreased rate constant for degradation (Kd) or both, and c) assess by kinetic analysis the effect of an acute addition of exogenous steroids to HTC cells on the catalytic activity of HMGR and its turnover. In addition, selected characteristics of purified HMGR from cells exposed to 25-hydroxycholesterol will be compared with the enzyme from unsuppressed cultures.

429. LIPID ESTERIFICATION IN LIVER AND MAMMARY GLAND

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This research project aims to explore esterification reactions for cholesterol and retinol in microsomal membranes and in cultured cells. Proposed experiments address the nature of the retinol esterification reaction in two tissues which are active in lipid metabolism and lipid secretion: liver and the lactating mammary gland. Using an assay based on the formation of H3-retinyl esters from H3-retinol, experiments will be conducted to describe the reaction and its dependence on specific cofactors. Experiments will also be conducted to determine the source of the fatty acid used in retinol esterification. These experiments will make use of lipid separations by high-pressure liquid chromatography and gas-liquid chromatography. The assays that will be developed for retinyl ester and cholesteryl ester synthesis will be applied in a set of experiments designed to compare these enzymatic processes in liver microsomes. Other studies seek to explore the distribution of cholesterol and retinol esterifying enzymes in membranous fractions of milk, and within the microsomal membrane itself.

Experiments are also proposed to compare the metabolism of cholesterol and retinol in isolated cultured hepatocytes and hepatoma cells. The primary objective of these studies will be to determine whether the uptake, esterification and intracellular storage of retinol in these cells are regulated in ways similar to those of cholesterol.

430. TRANSPORT IN CHOLESTEROL-RICH TUMOR MITOCHONDRIA

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We propose that meaningful correlations exist between: 1) the documented deregulation of feedback control over cholesterol biosynthesis in hepatomas, 2) the resulting endogenous enrichment of cell and organelle membranes with cholesterol and its esters, and 3) the potential alterations in the kinetic parameters of anion transport in tumor mitochondria. We are focusing attention on two critically important exchange transport processes in mitochondria; citrate and adenine nucleotide (ADP/ATP) transport. Transport kinetics will be studied by inhibitor-stop methods employing the specific transport inhibitors 1,2,3-denzenetricarboxylate (citrate) and carboxyatractyloside (adenine nucleotide). We shall investigate whether or not an observed rise (or other kinetic alteration) in citrate and ADP/ATP transport efficiency (Vmax, Vinitial, Km) occurs in two lines of Morris hepatoma, 3924A and 16, their hosts, and normal (control) mitochondria. We will correlate any changes noted in the transport kinetics with endogenous levels of cholesterol in the mitochondrial membranes of hepatomas. We will learn whether enrichment of the mitochondrial inner membrane with cholesterol constitutes a sufficient alteration in composition to effect both qualitative and quantitative shifts in citrate and ADP/ATP transport characteristics. This last question will be approached via a new method we have developed which permits the rapid transfer of cholesterol into the membranes of normal mitochondria in vitro, resulting in their becoming sterol-rich.

431. CHOLESTEROL IN NORMAL AND MALIGNANT LYMPHOCYTES

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The concentration of membrane cholesterol in normal or lenkemic lymphoid cells can be manipulated by subjecting the cells in vitro to oxygenated derivatives of cholesterol, which specifically depress the synthesis of sterols. Alternatively, whole animals are treated with such oxygenated sterols in the diet. The effect of such treatments upon specific immunological functions of lymphocytes, such as target-cell killing by T-cells and antibody production by B-cells will be investigated. In addition, the effects of dietary administration of oxygenated sterols, putative contaminants of the human diet, on cellular nutrition will be studied. Also, the morphological and physiological changes occurring in lymphoid tissues (lymph nodes, spleen, thymus) occurring upon dietary and parental administration of oxygenated sterols, will be characterized in young and old mice.

432. REGULATION OF STEROL BIOSYNTHESIS

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Using mutant strains of yeast, investigations are being conducted in the following areas: mechanisms of oxygenation of methyl groups at carbon-4 and carbon-14 of lanosterol; regulation of heme biosynthesis; membrane formation in dolichyl phosphate mutants; dependence on heme of hemoprotein synthesis; and the role of heme in synthesis and assembly of hemoproteins.

433. PHOTOCHEMICAL REACTIONS RELATED TO SKIN CANCER

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The role of ultraviolet light (UVL) and sunlight in the etiology of certain types of skin cancer seems well established. The mechanism(s) associated with these processes are not understood. It has been suggested that the carcinogenic activity of light is dependent upon the photochemical conversion of sterols to carcinogenic substances. The formation of a sterol-derived photoproduct with carcinogenic properties has now been demonstrated in UV-irradiated human and hairless mouse skin. The role of this compound, cholesterol alpha-oxide, in the etiology of UV-carcinogenesis is under investigation.

This proposal is designed to determine whether additional information will implicate the compound in the etiology of UV-carcinogenesis and involves: 1) Studies on the role of antioxidants in suppression of UVL-carcinogenesis; 2) studies on the metabolism and detoxification of the carcinogen; 3) the ability of the carcinogenic compound to transform normal cells, in cell culture, into cells with malignant potential; and 4) the ability of the carcinogen to induce tumors when applied, in vivo, to mice. Should the carcinogen induce transformation in cell culture and initiate tumors in vivo, then an overwhelming body of evidence implicating the compound in the etiology of skin cancer will have been amassed. Studies of the metabolism of this compound may indicate means by which its formation can be prevented.

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434. DIET AND CHOLESTEROL METABOLISM IN BREAST CANCER

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OBJECTIVES: To evaluate whether dietary and/or metabolic factors affect patterns of breast cancer development in general, and to identify which components of the diet are those which may affect breast cancer risk directly, or through metabolic changes. An attempt will be made to evaluate whether environmental dietary factors are more important than genetic metabolic factors in affecting cancer risk.

435. INTESTINAL REGULATION OF HEPATIC CHOLESTEROL SYNTHESIS (RATS)

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This is part of a broader project. A summary of this subproject is not available.

B. CHARACTERIZATIONS AND BIOSYNTHESIS OF GLYCOLIPIDS

436. FUCOLIPIDS - THEIR CHEMICAL CONSTITUTION AND ORGANIZATION IN SURFACE MEMBRANES OF NORMAL AND TUMOR CELLS

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Some human endodermal tumors and rat hepatomas accumulate various types of fucolipids, but some other tumors do not show significant accumulation of fucolipid. Only a few of the accumulated fucolipids have been chemically characterized such as alpha-L-fucosylceramide and fucosyllacto-N-tetraosylceramide; however, many of these structures have not been identified. It is possible therefore various fucosyltransferases are activated in some neoplastic tissue and some unusual fucolipids (such as fucosylceramide of human colonic carcinoma, fucoganglioside of rat hepatoma H-35) cells are synthesized which could be the distinctive cell surface markers for a particular type of malignant cell. Based on these backgrounds we propose to study: 1. A continuous chemical characterization of accumulated fucolipids in various types of tumors in vivo as well as in vitro. These include mouse epithelial cells transformed by murine sarcoma virus, and by chemical carcinogens, various types of human colonic carcinoma cells, rat hepatocarcinoma induced by feeding N-fluoro-acetamide (FAA), and rat colonic carcinoma induced by N-methyl-N'-nitro-N-nitroso guanidine. 2. Enzymatic basis of fucolipid accumulation in various tumors as above. 3. Studies on production of polyclonal and monoclonal antibodies directed to fucosylceramide, fucogangliosides and fucosyllacto-N-glycosylceramides. Their utilization for targeting of drugs to tumor cells through antibody-drug complex will be studied. This project is based on our recent success in production of monoclonal anti-glycolipid antibodies and in targeting drugs to glycolipids at the cell surface by antibody-drug complex.

437. IMMUNOSPECIFIC GLYCOLIPIDS OF NORMAL AND CANCER CELLS

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Tumor cells have been characterized by the phenotypes expressed on cell surface membranes. Previously, defective synthesis of glycolipids, lack of contact-dependent enhancement of glycolipid synthesis, and the change of surface glycosyl label, particularly the loss of 'galactoprotein a' and the appearance of 'ceramide X', were shown to be the characteristic phenotypes of transformed cells.

This proposal is for further studies on: (1) details of structural change and the mechanism of glycolipid synthesis and degradation in relation to control of cellular interaction, cell cycle, and cell proliferation; and the deficiency of such control mechanism on transformed cell membranes; (2) chemical and immunological characterization of surface-exposed glycolipids and glycoproteins (e.g., 'ceramide X' of NILpy cells and 'galactoprotein a' of NIL and BHK cells) in relation to immunogenicity of these surface components, and the use of surface specific components in studying immuno-suppression of tumor development and of blocking cell-mediated immunity; (3) surface modification of cells and concurrent changes of cell physiology by applying purified anti-glycolipid antibodies and by an artificial enrichment of a special glycolipid in membranes; a possible control of tumor cell growth through such surface modification will also be studied; (4) comparative surface profile as to glycolipids of genetically related cells showing high and low tumorigenicities and/or high and low metastatic properties.

The long-term goal of these studies is to determine the role of cell surface heteroglycans which ultimately may provide clues for the control of cancer.

438. GLYCOLIPIDS OF NORMAL AND TRANSFORMED MOUSE LYMPHOCYTES

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There has been much speculation in recent years as to the role of glycosphinogolipids as functional constituents of the plasma membrane of mammalian cells, especially in transformed cells, because of the many changes in glycolipid content which have been observed. There is, however, little information on the exact physiological properties of individual glycolipids or on the role that they play in transformation.

Lymphocytes from the T lineage in mice contain at least 12 gangliosides most of which are insusceptible to Vibrio Cholera sialidase. This indicates that the sialic acid in these molecules is in an internal linkage where it is protected. This neuraminidase treatment produces no lactosylceramide, lactoneotetraosylceramide, or GM1, which are the products of hydrolysis of most common gangliosides. Only small amounts of asialo GM1 are produced which indicates that there is a terminally sialated derivative of asialo GM1 in lymphocytes.

The beige mouse has been shown to have defect in natural killer activity, which is thought to be an important anti-tumor mechanism. These mice have altered levels of both asialo GM1 and its sialosylated derivative while most of the other gangliosides and glycolipids remain at normal levels. Analysis of the structure of these possibly significant molecules is underway.

439. ROLE OF GLYCOLIPIDS IN IMMUNE CELL FUNCTION

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The overall objective of this project is to determine the composition and role of specific glycolipids in immune effector cell function. Two major aspects will deal with: (1) Testing whether certain glycolipids can function as tumor-specific cell surface markers. We have defined the glycolipid gangliotriosyl ceramide (asialo GM2) as a potential tumor associated marker in two mouse cell lines and have demonstrated both in vivo and in vitro that anti-asialo GM2 specific antibody-dependent immune lytic functions can be directed to the tumor cells. Future experiments will be designed to develop antibody-independent cellular cytotoxicity specific for asialo GM2. (2) Defining glycolipid markers on immune system effector cells. We have recently described the glycolipid ganglio-N-tetraosylceramide (asialo GM1) as a marker for murine natural killer (NK) cells. In addition we are determining the chemical structures of glycolipids on NK-susceptible target cells to define the 'target antigen' to which NK cells are directed. The next step will be to determine what role effector cell glycolipids play in lytic phenomena. A vital class of reagents for these studies are monoclonal anti-glycolipid antibodies produced by the hybridoma technique.

440. IMMUNOREGULATORY PROPERTIES OF HUMAN GANGLIOSIDES

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This project tends to examine the molecular and cellular basis on the immunosuppression by specific gangliosides. The studies will determine if exogenous gangliosides are incorporated into lymphocyte membranes and if specific gangliosides are synthesized during activation.

Whether specific gangliosides inhibit distinct lymphocyte subpopulations will be determined by a number of in vitro assays of lymphocytes function currently operational in our laboratories.

441. GLYCOSYLATION MECHANISMS OF CELL LIPIDS AND PROTEINS

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The overall objective of this research is to study the structure, biogenesis and function of animal cell surfaces. This interest has been stimulated by recent observations relating cell surfaces with regulation of cellular growth. The specific purpose of this project is to understand the mechanism(s) of glycosylation (with particular emphasis on sialic acid) of glycolipids and glycoproteins. We will continue and extend to other sugar nucleotides our current studies on the subcellular site(s) of synthesis of CMP-sialic acid. Attempts will be made to determine how sugar nucleotides get from their intracellular site(s) of synthesis to the site(s) of sugar transfer. We will further pursue our recent observations on the uptake by cells of sialic acid and the subsequent incorporation of this sugar into glycoproteins and lipids. The intracellular site(s) where the covalent binding of sialic acid to glycoproteins and glycolipids occurs will be studied by pulse-chase kinetics with cells in tissue culture and mouse liver. Although initial experiments will monitor the glycosylation of a mixture of glycoproteins and lipids, futher studies will concentrate on one particular glycoprotein. The subcellular movement of covalently-bound label will be monitored by a combination of subcellular fractionation and electron microscopy-autoradiography. These studies with normal cells will be extended to cells in tissue culture infected with a virus that is temperature sensitive for transformation since such a system should enable us to distinguish transformation related phenomena from ancillary ones.

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442. GLYCOLIPID METABOLISM IN TUMOR AND TRANSFORMED CELLS

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The major objective of the proposed research in the immediate future are two-fold: 1) Isolation, characterization and biosynthesis of glycosphingolipids of the globoside family in guinea pig embryonic 103 cells and their chemically transformed variant 104Cl cell. Differential binding of 125I-labeled lectins (Dolichos biflorus and Bandeiraea simplicifolia) and toxins (cholera and ricin) to 103, 104Cl and 106B cell surfaces under various growth conditions. 2) Studies on the structure and biosynthesis of N-acetylglucosamine containing blood-group related neutral

glycosphingolipids in mouse neuroblastoma N-18 and human neuroblastoma IMR-32 cells. Differential binding of 125I-labeled lectins (Ulex europeus, Euonymus europeus and Lotus tetragonolobus), cholera toxin, and ricin to IMR-32 and N-18 cell surface before and after chemically induced (6-MG, BrdUrd and (But)2cAMP) differentiation.

The glycosphingolipids are highly enriched in the plasma membranes of eukaryotic cells. Alterations in their composition and modification of their glycose units might affect intercellular relationship. Our preliminary observations and the proposed studies should provide insight into the cell-surface glycosphingolipid alterations during chemical transformation of an embryonic cell line (103 vs 104Cl) and during chemically induced differentiation of tumor cells (N-18 and IMR-32). Furthermore, we hope to compare the structures of glycosphingolipids present on the surfaces of tumor cells of two different species (mouse N-18 vs. human IMR-32).

443. GLYCOSPHINGOLIPID METABOLISM AND TUMORIGENESIS

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This proposal is for continuing support to elucidate and characterize one biochemical lesion accompanying tumorigenesis in mammalian cells. We will investigate progressive alterations of gangliosides of the cell surface. Ganglioside changes correlate with alterations of specific glycosyl transferase activities of ganglioside biosynthesis. The latter are membrane-bound enzymes found with the cell's internal membranes (endomembranes). New work will focus on the regulatory events associated with modulations in glycosyl transferase activities of these internal endomembranes. Early events will be studied with emphasis on the enzymes that catalyze synthesis of precursor glycolipids, enzymes at major branchpoints in the ganglioside biosynthetic pathway, synthesis of sugar nucleotides, and enzymes related to synthesis of sugar nucleotides. Experimental materials will be principally liver tumors of the rat (primary and transplantable) induced with the chemical carcinogen N-2-fluorenylacetamide. Major findings will be verified in induced and transplanted mammary carcinomas and in cell lines derived from these sources and growing in monolayer cultures.

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444. MAMMALIAN CELL SURFACE CARBOHYDRATE-BEARING COMPONENTS

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OBJECTIVE: To sort out the molecular basis of eucaryotic cell surface glycolipid and glycoprotein behavior, interactions, and response to external stimuli.

APPROACH: Various glycolipids ranging from galactocerebroside to gangliosides, as well as the human erythrocyte glycoproteins, glycophorin and the concanavalin A receptor, are being studied. The basic approach involves isolation of the carbohydrate-bearing species of interest for investigation in systems of controlled complexity. The systems may be simple solutions, lipid bilayers, or intact cells. The behavior of the species in question is monitored by microscopy, covalently attached spin labels, or immunological techniques.

445. ROLE OF GLYCOCONJUGATES IN FORMATION OF METASTASIS

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The elucidation of the possible relationship between glycoconjugate levels and profiles (gangliosides in particular) of two rat mammary tumors (SMT-2A and MT-W9a) and the formation of metastasis is the goal of this project. SMT-2A tumor metastasizes very extensively to different organs in rats, including lungs, whereas MT-W9a does not metastasize under experimental conditions. It has been observed that SMT-2A contains about 3.5-fold more lipid-bound sialic acid than nonmetastasizing MT-W9a tumor per 100 mg of lipid extracted. Also, the ganglioside pattern of these two tumors is different. SMT-2A tumors contain relatively higher quantities of two disialogangliosides, GD2 and GD1b, trisialoganglioside GT1b and tetrasialoganglioside GQ1b. On the other hand MT-W9a contains relatively and absolute more monosialohematoside GM3. The relative amounts of monosialoganglioside GM1 and disialohematoside GD3 are also higher in MT-W9a.

Analyses of gangliosides from lungs of SMT-2A-bearing rats (54 days postimplantation), containing large numbers of macroscopic metastatic nodules, reveal that the level of lipid-bound sialic acid may be up to 4-fold higher than in normal lungs and may be even higher than in SMT-2A primary tumors. The level of lipid-bound sialic acid in larger excised lung nodules (occasionally found) and secondary tumors from axillary lymph nodes are approximately 2-fold higher than in primary SMT-2A tumors. All this very strongly suggests that primary tumor cells (or clumps of cells) with a relatively higher content of gangliosides than average may more effectively originate metastatic (secondary) tumors.

Investigation of the content and profile of gangliosides in isolated tumor plasma membranes shows that plasma membranes are enriched in total ganglioside content as compared with whole cells. The degree of enrichment in SMT-2A plasma membranes is 1.8-fold and in MT-W9a, 3.1-fold. The total ganglioside content in plasma membranes from metastasizing tumor SMT-2A is 1.8-fold higher than in plasma membranes from nonmetastasizing tumor MT-W9a.

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446. ROLE OF GANGLIOSIDES IN THE PATHOGENESIS OF GLIOMAS

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The long-range goal of this project is to understand at the molecular level the pathogenetic mechanisms responsible for the uncontrolled proliferation of human gliomas. Current evidence indicates that the cell surface plays a critical role in normal cellular growth control. Gangliosides are a class of glycolipids located at the cell surface which probably participate in several biological functions one of which may be the control of cell division. Ganglioside compositions different from parent tissues have been found in many tumor types, including gliomas, which may be related to their lack of cellular growth control. Gangliosides appear to be shed from the surface of glioma cells into the blood and evidence from our laboratories and others indicates that gangliosides suppress the immunoreactivity of peripheral blood mononuclear cells. Thus, it is possible that these shed gangliosides may be responsible for immunodeficiencies in patients with gliomas. In this proposal three groups of experiments have been designed to investigate the roles of gangliosides in phenomena relevant to the pathogenesis of human gliomas.

In the first group we will study aspects of ganglioside metabolism in cultures of human glioma and normal glial cells under different growth conditions to determine if their ganglioside metabolism is related to cell density and/or cell multiplication. Comparisons between normal and neoplastic glia will be made.

The second group includes experiments to investigate the effects of exogenously added gangliosides on several biological functions relevant to cell multiplication of normal and neoplastic cultured glial cells.

The third group of experiments has been designed to investigate whether or not gangliosides in human gliomas and serum of patients with gliomas are capable of suppressing the reactivity of human normal peripheral blood mononuclear cells to nonspecific mitogens.

447. EFFECT OF MALIGNANT TUMORS UPON BLOOD PLASMA GLYCOSPHINGOLIPID PATTERNS

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This is part of a broader project. A summary of this subproject is not available.

C. BIOSYNTHESIS AND METABOLISM OF PHOSPHOLIPIDS AND GLYCEROLIPIDS

448. STRUCTURAL CHARACTERIZATION OF MEMBRANES

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Our goal is to characterize the molecular structure and conformations of the lipids in their various physical states (i.e., liquid-crystal, gel, etc). In particular, we will focus on those changes which are related to the changes in the fluidity of the bilayer as a function of varying temperature, water content and other extrinsic factors. The technique we use is primarily laser Raman spectroscopy, which has been shown to be a sensitive probe of both molecular conformation and the physical state of the lipids. We are presently using phosphatidyl cholines that have been selectively deuterated at specific positions along the hydrocarbon chains to monitor the fluidity of the bilayer at different depths from the polar head group as a function of temperature. By this means we can monitor both the average fluidity and the local order of the membrane. Our results show that the membrane gets progressively more fluid as the distance from the polar surface increases and that Raman spectroscopy is sensitive to the interaction of the polar group with the hydrocarbon chain.

By incorporating other molecules such as cholesterol or antibiotics (e.g., valinomycin) into the deuterated phospholipid bilayers one can monitor the interaction of lipid with the incorporated molecule as a function of distance from the polar group.

We also propose to extend these studies to glycolipids which have been deuterated either at specific positions in the hydrocarbon chain region or on the sugar moiety. Using this technique we plan to investigate the details of the molecular interaction of these two components of the glycolipid as well as to determine the orientation of the sugar moiety relative to the bilayer plane.

449. THE MECHANISM OF SPHINGOMYELIN SYNTHESIS AND ITS ROLE IN MEMBRANE BIOGENESIS

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The objective of this research project is the identification of the proximal source of the phosphocholine moiety of sphingomyelin, and subsequent purification of the enzyme that converts ceramide to sphingomyelin. The roles of CDP-choline, dCDP-choline, and phosphatidylcholine as sources of phosphocholine will be evaluated. We will address this problem using primary hepatocyte cultures and microsomal enzyme preparations from liver and kidney. Studies with hepatocytes will exploit the N-methylation pathway for phosphatidylcholine synthesis to selectively label the phosphatidylcholine pool without signficantly labeling the cytosine nucleotide pool. Pulse-chase experiments with radiolabeled choline will also be conducted, and the specific activities of each of the potential precursors will be measured. Experiments with microsomal preparations will employ lipase-generated, membrane-bound ceramide as substrate, to avoid the problems inherent in adding water-insoluble substrates to reaction mixtures. By manipulating the microsomal membrane content of ceramide and phosphatidylcholine we will have a second method for determining the importance of each of the potential substrates. The combination of these two approaches will demonstrate which pathway for sphingomyelin synthesis is the physiologically important one. Following the identification of the synthetic pathway we will attempt to purify and characterize the enzymes involved in the terminal step of sphingomyelin synthesis.

450. UNUSUAL LIPIDS IN CANCER TISSUES

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Membrane changes associated with neoplastic transformation will be defined in molecular terms and will be correlated with membrane and cell surface expression relative to differentiation and degree of malignancy. Special attention will be focused on the role of lysolecithins and lysolecithin-type cholinephospholipids derived from short-chain diols in various membrane, enzyme and cell processes, and on the controlling steps that maintain physiological levels of monochain phospholipids. The effects of lysolecithins and diol-derived phosphocholines will be studied in tumors grown in rodents and in cultivated Novikoff rat hepatoma cells, virally transformed Balb/c 3T3 A31 cells, cells from human tumors, and others, as well as in respective controls. The effect of monochain phospholipids on membrane and surface properties of normal and transformed cells will be defined in terms of their susceptibility to lysis, and in terms of membrane fluidity as expressed by 2H, and 13C and 31P relaxation measurements and fluorescence spectroscopy. Drug-mediated lecithin inhibition and lysolecithin stimulation will be evaluated as a means to affect cell surface properties and to possibly influence cancerous proliferation. The role of monochain phospholipid-cholesterol-lecithin interactions will be studied in biological and model membranes.

BIBLIOGRAPHIC REFERENCES: K. Hayase, S. Parthasarathy, C. M. Eppler and W. J. Baumann. A rapid assay of acyl-coenzyme A:lysolecithin acyltransferase activity. J. Lipid Res., 21, 484-488 (1980). S. Parthasarathy, R. Murari, K. S. Crilly, and W. J. Baumann. Modulation of diacylglycerol acyltransferase by lysophosphatidylcholine and related monochain phospholipids. Biochim. Biophys. Acta. 664, in press (1981).

451. THE INFLUENCE OF AGE ON PHOSPHATIDYLINOSITOL TURNOVER

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OBJECTIVE: The objective of this project is to study the turnover of phospholipids in proliferating keratinocytes and fibroblasts in culture.

APPROACH: Keratinocytes cultured on lethally irradiated 3T3 fibroblasts will be exposed to 12-0-tetradecanoylphorbol-13-acetate (TPA) and to epidermal growth factor (EGF). Fibroblasts in culture will be exposed to TPA and to EGF. Alterations in cell phospholipids will be studied with the use of radioisotopes and thin layer chromatography.

452. METABOLISM, REGULATION, AND FUNCTION OF ETHER-LINKED GLYCEROLIPIDS AND THEIR PRECURSORS IN CANCER CELLS

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Levels of glycerolipids that contain ether linkages at the sn-1 position are elevated in cancer cells in comparison to normal tissues. A major aim is to elucidate the enzymatic control points and factors responsible for the regulation of ether- and ester-type glycerolipids in squamous cells (B2-1), Ehrlich ascites cells, or L-M cells grown in monolayer and suspension cultures. The cellular level of ether lipids in such cells can be manipulated by supplementation of glucose of alkylglycerols to the growth media. Enzyme activities, reactants, and related factors involved in the metabolism of ether lipids will be correlated with the level of ether lipids to determine what steps are rate-limiting. We are especially interested in the key enzymes that form the alkyl linkage and intermediates. The enzymes receiving highest priority in these studies are alkyl synthase (substitutes an alcohol for the acyl group of acyldihydroxyacetone-P to form alkyldihydroxyacetone-P, the first detectable ether-linked intermediate), acyl-CoA reductase (converts fatty acids to the corresponding alcohol), the transferases that channel alkylacylglycerols into neutral and polar lipids, and alkyl desaturase (forms plasmalogens). The alkyl and alk-1-enyl ether cleavage enzymes, fatty alcohol: NAD ion oxidoreductase (oxidizes the alcohol to the corresponding acid), the lysophospholipase D will also be

investigated under conditions that yield different membrane levels of ether lipids; these latter enzymes appear to have very importa- catabolic roles in the regulation of ether lipids in normal cells. We plan to determine the membrane topography of these and related enzymes and their products in microsomes from cancer cells; comparisons, when possible, will be made with those already established for liver. The role of ether lipids in membranes will be evaluated by determining how the ether lipid content of membranes affect the functional activities of important marker enzymes in specific organelles. These experiments will focus on enzymes involved both in intermediary metabolism and in transport processes. Pilot experiments with a newly identified acetylated ether lipid class that possesses profound biological activities will be intiated with HSDM1C1 fibrosarcoma cells. These will concern the effect of 1-alkyl-2-acetoyl-sn-glycero-3-phosphocholine on growth kinetic (Text Truncated - Exceeds Capacity)

453. ETHER LIPIDS IN CANCER-ENZYME MECHANISMS

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Recent progress has included the solubilization of alkyl synthase from microsomes using detergents and phospholipase A2 or C. Since this enzyme catalyzes the first committed step in ether bond formation, purification of the alkyl synthase will permit us, for the first time, to assess the unique reaction mechanism that substitutes a long chain fatty alcohol in toto for the acyl group of acyl dihydroxyacetone-P to form the alkyl ether linkage. The properties of alkyl synthase will be characterized an an antibody prepared for use in investigating its regulation and firmly establishing its exact subcellular site. Acyl-CoA reductase (forms the alcohol precursor) and Delta-alkyl desaturase (forms plasmalogens) will be characterized using similar approaches. The ultimate objective is to understand how these three crucial enzymes in the ether lipid pathway function and how they are regulated in cancer cells and glandular tissues that are enriched in ether-linked lipids.

BIBLIOGRAPHIC REFERENCES: Lee, T-c., Fitzgerald, V., Stephens, N., and Snyder, F. Activities of enzymes involved in the metabolism of ether-linked lipids in normal and neoplastic tissues of rat. Biochim. Biophys. Acta 619, 420-423, 1980. Cabot, M.C. and Snyder, F. Manipulation of alkylglycerolipid levels in cultured cell: fatty alcohol versus alkylglycerol supplements Biochim. Biophys. Acta 617, 410-418, 1980.

454. O-ALKYL LIPIDS IN SURFACE MEMBRANES OF TUMOR CELLS

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Neoplasms contain increased quantities of O-alkyl lipids. In order to detect surface membrane abnormalities in malignant versus non-malignant tumors, we are measuring the O-alkyl lipid content in these membranes by a procedure which isolates these structures as vesicles from a microsomal preparation. O-alkyl lipids of several tumors have now been quantitated by photo densitometry. We have measured O-alkyl and alk-1-enyl lipids of surface membranes of several metastasizing and non-metastasizing tumors. Work now in progress concerns the measurement of a larger series of metastasizing and non-metastasizing tumors.

The mechanism leading to increased O-alkyl lipid synthesis in Erlich ascites tumor cells may involve a decrease in long chain fatty alcohol dehydrogenase activity. Thus, fatty alcohol metabolism may be shunted to O-alkyl lipid synthesis. It is also possible that fatty alcohol synthesis from fatty acids may be increased in neoplasms. We have recently obtained data on alcohol dehydrogenase activity in several solid tumors and compared these results with alcohol dehydrogenase activity in normal tissues. Further work will be necessary before any conclusions can be drawn.

We have published evidence to show that the O-alkyl lipid bond is formed by the intermediacy of an endiol of acyl-dihydroxyacetone-phosphate. In the absence of fatty alcohol, this mechanism requires the formation of dihydroxyacetone-phosphate which has exchanged the pro-R hydrogen, and the release of fatty acid which has retained both carboxyl oxygens. We have recently demonstrated the release of dihydroxyacetone-phosphate which has exchanged the pro-R hydrogen and we are currently investigating whether or not the fatty acid which is released contains both carboxyl oxygens.

BIBLIOGRAPHIC REFERENCES: Friedberg, Samuel J. and Gomillion, Marc: Hydrogen exchange in the formation of dihydroxyacetone phosphate from acyl dihydroxyacetone phosphate in O-alkyl lipid synthesis in Ehrlich ascites tumor cell microsomes. J. Biol. Chem. 256:291-295, 1981.

D. OTHER STUDIES ON LIPID METABOLISM AND BIOSYNTHESIS IN NORMAL AND TUMOR CELLS

455. RELATIONSHIP BETWEEN LIPID MOLECULES OF PLASMA MEMBRANES AND SURFACE PROPERTIES OF NORMAL AND VIRALLY TRANSFORMED CELLS

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OBJECTIVES: 1) To characterize the lipid structure of virally transformed cells. 2) To gain insight into whether increased lectin-mediated agglutinability of transformed cells is related to lipid changes of cell surface. 3) To establish requirement of essential fatty acids for growth of virally transformed vs normal cells. 4) To investigate the role of different gangliosides in growth control.

APPROACH: 1) Lipid structure will be examined at the level of individual lipid classes and molecular species. 2) The relationship between lipids of cell surface and susceptibility to concanavalin A will be studied by enriching plasma membranes of BALB/c3T3 cells with different phosphatidylcholine molecular species. 3) Requirement of essential fatty acids for growth of virally transformed cells and normal cells will be evaluated by growing these cells in media containing lipid-depleted serum. 4) Growth regulatory function of GM3 ganglioside will be ascertained by evaluating the effects on growth properties of BALB/c3T3 and SV3T3 induced by incorporation of GM3 and treatment with anti-GM3 antibodies.

PROGRESS: 1) Analyses of ether-linked lipids of BALB/c3T3, SV3T3 cells revealed that transformation by SV40 virus is associated with an accumulation of alk-1-enyl phosphatidylcholine and with changes in the alk-1-enyl group composition of alk-1-enyl phosphatidylcholine and phosphatidylethanolamine. 2) Incorporation of dioleoyl lecithin increased agglutinability of 3T3 cells, while no effect was observed with the divaccenoyl- or dipalmitoleoyl lecithins. This observation indicates that influence of dioleoyl lecithin on agglutinability is specific to its structure. 3) Growth in the presence of lipid-depleted serum induced a greater reduction of saturation density in SV3T3 cells than in 3T3 cells. However the exposure to lipid-depleted serum to which lipids had been restored induced changes in saturation densities of SV3T3 and 3T3 cells similar to those found in these cells grown in lipid-depleted serum. 4) Preparation of purified GM3 from liver is to be concluded.

456. MEMBRANE LIPID ASYMMETRY IN A TUMORIGENIC CELL LINE

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Although there exists extensive information indicating that membrane alterations are involved in the neoplastic process, very little is known concerning the role of phospholipid and sterol alterations in neoplasia. Despite recent advances in understanding of the chemical, physical and molecular properties of lipids in model systems, there is a considerable lack of information correlating these properties with those of normal and neoplastic systems. Almost nothing is known of processes such as phospholipid and sterol exchange that regulate lipid composition in cancer cell membranes, the asymetric distribution of lipids in cancer cell membranes, the regulation of membrane lipid asymmetry, or the possibility of biophysical as well as chemical asymmetry existing in cancer cells.

The long-term goals of this proposed research are to provide an in depth study of surface membrane lipid asymmetry in a tumorigenic cell line as follows; 1) The existence of membrane

lipid asymmetry in a cell line grown in the absence of serum or hormone supplements will be tested in order to determine if membrane lipid asymmetry can be of de novo origin rather than being imposed on the cell by serum components. 2) The regulation or manipulation of membrane lipid asymmetry will be studied by supplementation of the LM cells with different fatty acids, polar acids, polar head groups, or sterols of both natural origin or synthetic analogues. 3) The possible existence of asymmetric distribution of membrane lipid biophysical properties will be established. 4) The effect of membrane lipid asymmetry alterations on some biological properties of LM cells will be surveyed. The results of these investigations should considerably broaden the scope of knowledge on the role of membrane lipid asymmetry in the neoplastic cell membrane.

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457. TUMOR LIPIDS - METABOLISM AND STRUCTURAL STUDIES

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The major objectives of the project are to determine whether abnormalities in the lipid structure or metabolism exist between normal and neoplastic tissue. The possibilities of regulating lipid metabolism as a means of controlling neoplastic growth are also being assessed. Studies will continue to determine the molecular basis for the observed loss of specific oleate to vaccenate ratios in the hepatoma lipids that characterize the lipid classes of normal liver. Generally, the approach will involve the use of metabolic analogues and specific inhibitors to change the level of oleate and vaccenate. Experiments are also planned to elucidate at the molecular level the early changes that occur in the lipid metabolism of the host animal soon after tumor transplantation. We will seek to determine the origin of the unusual hexadecenoate positional isomers that occur at relatively high levels in the hepatoma, but are virtually absent from normal liver. Lipids from subcellular organelles of hepatoma cells will be analyzed at the molecular species level and compared with the corresponding membrane fractions from normal hepatocytes.

458. REGULATION OF FATTY ACID BIOSYNTHESIS IN MAMMARY TUMORS

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Our earlier results of immunotitration studies had suggested that acetyl CoA carboxylase isolated from transplantable mammary tumors (R3230 AC and 13762) was different from the enzyme obtained from rat lactating mammary gland. This conclusion was based upon the observations that neutralization of carboxylase activity present in the cellular extracts of tumors required much higher amounts of anti-acetyl CoA carboxylase than was needed to inhibit an equivalent amount of the carboxylase activity of normal tissue origin. Recently a number of laboratories have provided evidence suggesting that acetyl CoA carboxylase may be regulated by phosphorylation/dephosphorylation mechanism. The dephospho form of the enzyme is believed to be active whereas the phospho form is less active. In view of these observations, we want to determine whether the carboxylase of neoplastic origin mainly exists in a modified form which is enzymatically less active. Similary, we are attempting to isolate and characterize the converter enzymes from rat mammary gland that add and subtract phosphoryl group(s) from the highly purified acetyl CoA carboxylase.

We are also continuing our studies on the mechanism of inhibition of fatty acid synthetase by S-(-4-bromo-2,3-dioxobutyl)-CoA.

BIBLIOGRAPHIC REFERENCES: Gratzner, H. G., Ahmad, P.M., Zegadlo, J. and Ahmad, F., Immunofluorescent Localization of Acetyl CoA Carboxylase, Fatty Acid Synthetase and Pyruvate Carboxylase During the Adipocyte Conversion of 3T3 Fibroblasts, Cell Biol. Int. Reports, 4:497-508 (1980).

459. METABOLISM OF HEPATIC TRIGLYCERIDE-RICH VESICLES

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The broad objectives of the proposed research are to gain insight into the mechanisms by which the biogenesis and mobilization of membrane-rimmed triglyceride-rich vesicles are regulated in the liver cell (hepatocyte). Particular emphasis will be placed on the modulatory actions of insulin, a lipogenic hormone, and glucagon and cAMP, anti-lipogenic agents, at the molecular level. A hormone-sensitive, chick hepatocyte primary culture system developed in the sponsor's laboratory will be employed as an in vivo model with which lipid metabolism can be studied in a controlled 'physiological' setting. The objectives are to determine the intracellular origin, biogenesis, and mechanism of assembly of cytoplasmic membrane-bounded triglyceride rich vesicles; to define the mechanism and its control whereby vesicular triglyceride is mobilized; and to investigate the acute control of hepatocyte fatty acid synthesis and metabolism through characterization of the mechanism by which cAMP (and glucagon) activate the re-entry of vesicular triglyceride into cellular metabolism and stimulate ketogenesis from mobilized fatty acid. The proposed studies form part of the general goal of the proponent and the sponsor's laboratory to determine the mechanisms by which the synthesis and metabolism of fatty acids, triglyceride, and very low density lipoprotein are regulated in the liver cell

460. GLUCOSE AND FAT METABOLISM IN DIABETES, CANCER AND AGING

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Ten manuscripts in cancer, diabetes, molecular biology (liver membrane synthesis), and atherosclerosis research were accepted for publication based upon the last several years' work. Turnover of different VLDL-, LDL-, and IDL-apoB containing particles has been studied in rats and an attempt made to integrate these data with the complex pattern of VLDL-TG and VLDL-remnant-TG. An unusual pattern of VLDL-TG turnover was found in rats fed fat-free diets (58% fructose or 58% glucose). Manuscripts are in preparation. Heterogeneity of VLDL-apoB in various animal species was studied; a unique pattern was found in rats and mice. Studies of phospholipid FA turnover in the membranes of aging adipocytes was terminated because incorporation of labeled FA into adipocyte PL was too slow, non-linear and difficult to quantify, especially with the variable but highly significant contamination due to associated non-adipocytes in adipose tissue. However, techniques developed in the course of this work are being applied to studies of hormone action, kipogenesis, cancer nutrition, and aging adipose tissue. A model was developed to account for disparate rates of fatty acid oxidation by Ehrlich ascites cancer cells in vivo using (9, 10-3H)- and (1-14C)-labeled fatty acids. Re-evaluation of fatty acid needs of the tumor for oxidation and growth was necessitated. Improved studies of plasma VLDL-TG turnover and transport to cancer cells in mice were done with doubly-labeled TFGA and intergrated with the new oxidation data into a revised model of tumor-host fatty acid metabolism.

461. ONCOLYTIC EFFECTS OF LIPOPROTEINS

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A very low density lipoprotein (VLDL, Sf 20 - 400) associated oncolytic factor, isolated from rat serum, has been examined. An in vitro colony-inhibition assay which employs the rat prostate adenocarcinoma (PA-II and III) as target cells has been developed to quantitate the oncolytic potency of VLDL. The mechanism of oncolysis is under investigation. VLDL has been further characterized by thin-layer chromatography, affinity chromatography, radiolabelling, and sequential ultracentrifugation. The various subclasses of VLDL have been separated, and

most of the oncolytic activity was recovered in the Sf 100 - 400 fraction.

The surveillance role of VLDL in host defense against neoplasia has been studied in relation to tumor metastasis and chemical carcinogenesis.

BIBLIOGRAPHIC REFERENCES: Chan, S.Y. and Pollard, M. In Vitro Effects of Lipoprotein-associated Cytotoxic Factor on Rat Prostate Adenocarcinoma Cells. Cancer Res. 38:2956-2961, 1978.

462. KINETIC MODELING OF HUMAN PLASMA LIPOPROTEIN METABOLISM

Berman, M., Foster, D., Riemke, R., Schwartz, C., Grundy, S., Beltz, W., Malmendier, C., Fisher, W., Hazzard, W., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Mathematical Biology Section, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

Kinetic models of plasma apoproteins, cholesterol and triglyceride are being constructed based on data from experiments in man. The models are used to integrate plasma lipoprotein interactions with enzymes and receptors and to provide a better understanding of plasma lipoprotein synthesis and metabolism in health and disease. The models are particularly useful for the rigorous testing of hypotheses, the design of future experiments, and in quantitating the effects of various perturbations.

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463. MEMBRANE INTEGRITY AND CYCLOPROPENOID FATTY ACIDS

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In the proposed project rainbow trout will be reared on diets containing various levels of cyclopropenoid fatty acids. Plasma and cytoplasmic liver cell membranes will be isolated and characterized chemically and by electron microscopy. Isolated membrane fractions will be studied to determine the relationship between cyclopropenoid fatty acid induced lipid alterations and (1) the structure of membrane lipids, (2) the functioning of membrane-bound transport enzymes, (3) the topography of the plasma membrane, and (4) the functioning of hepatic drug-metabolizing enzymes.

These studies will provide information on the mechanism by which dietary cyclopropenoid fatty acids as cocarcinogens affect the functioning of cellular membranes. Moreover, these data should help to assess the possibility of using this system as a means of studying the relationship between altered membrane lipids and cocarcinogenicity.

464. EFFECT OF LIPID MODIFICATION OF HEPATOMA CELLS ON IMMUNE CYTOLYSIS

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PROJECT OBJECTIVES: The specific aims are to 1) modify fatty acid composition of the tumor cell membrane and study in detail the effects of these changes on membrane lipid composition and structure; 2) observe the effect of these changes on immunocytolysis (e.g., complement dependent cytotoxicity and natural killer activity); and 3) study the physical state of receptor molecules (e.g., tumor antigens) on fatty acid modified cell membranes. The long term goal of this proposal is understanding immunocytolysis as a function of membrane phospholipid composition, membrane fluidity, and the physical state of cell membrane receptor molecules.

PLAN AND METHOD: These studies wll be conducted by modifying rat hepatoma cells in vitro with linoleic, oleic, linolenic, palmitic, stearic and arachidonic acid. Fatty acid composition as well as phospholipid and cholesterol contents of the cell membrane will be analyzed, and these modified cells will be subjected to immocytolysis by antibody and complement, and killing by splenic natural killer cells. Fluorescence probes, electron spin resonance and radioimmunoassay will be used to detect the physical states of receptor molecules (tumor antigens) and membrane fluidity in the fatty acid modified tumor cells in an attempt to delineate some of the mechanisms involved in cell lysis.

SIGNIFICANCE: Our studies would have a dramatic effect on current

465. MAGNETIC RESONANCE STUDY OF PROTEIN-LIPID INTERACTIONS

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This project will be concerned with delineating the thermodynamical, kinetic and structural aspects of the interaction of an intrinsic membrane protein, mitochondrial cytochrome c oxidase, with lipids. Dahlquist and coworkers have already been able to reconstitute cytochrome oxidase with either 2H labelled or 19F labelled lipids and observe the 2H or 19F NMR spectra of these particles. These studies (Proc. Natl. Acad. Sci. (1977) 74, 5435; Biochemistry (1977) 16, 5746) show the presence of two distinct environments corresponding to the free lipid and that lipid which interacts with the protein. Now that the technical problems associated with preparing the reconstituted particles and making the measurements have been mastered, it seems that the tools are in hand to examine a number of questions concerning the interactions between proteins and lipids. These include: examination of packing and mobility of lipid sequestered about a protein; whether certain lipids are preferred by some membrane proteins as opposed to other lipids; whether protein segregations can be mediated by lipid content and composition; whether different proteins interact with lipids in different ways or if a single picture for how a hydrophobic protein interacts with lipids can be developed. While a number of approaches (including calorimetry) will be used, NMR will provide the bulk of the information required to answer these questions.

466. MEMBRANE COMPOSITION AND HYPERTHERMIC CELL DEATH

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Recent interest in the use of hyperthermia in cancer therapy has brought about a need for increased understanding of its effects on cells. Earlier workers postulated that cell lipid viscosity was the determining factor in a cell's response to temperatures. The studies proposed will undertake to systematically study the effect of varying membrane lipids, cell microviscosity, and survival at elevated temperatures. An unsaturated fatty acid auxotroph K1060, of E. coli, yields membranes of differing lipid composition and properties that depend on growth conditions (unsaturated fatty acid supplied and growth temperature). Cell microviscosity will be studied using the polarization properties of a fluorescent probe molecule dissolved in the membrane. Cell survival will be determined on cells held at a graded series of temperatures. These data will be modeled to provide an estimate of the relative role of each factor in hyperthermic killing.

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467. EFFECT OF ESSENTIAL FATTY ACID DEFICIENCY ON BIOCHEMICAL AND BIOLOGICAL PARAMETERS OF ASCITIC SARCOMA 180

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OBJECTIVE: To determine the effect of essential fatty acid deficiency on certain biochemical and biological parameters of ascites sarcoma 180.

APPROACH: Swiss rats are fed diets deficient in essential fatty acids for 50 days and then sarcoma 180 ascites cells are injected into the peritoneal cavity. Measurements are made of time for cell duplication, lipid and fatty acid composition of those lipids of cells and livers of host rats.

The incorporation of 114C linoleic acids is also measured, incubating cultured cells in animals deficient in essential fatty acids and in normal animals.

PROGRESS: In accordance with the results, the ascites sarcoma 180 cells will be independent of the essential fatty acids available for their development. (Translated)

468. LIPID LEVELS AND CHOLESTEROL METABOLISM IN RELATION TO HUMAN BREAST CANCER RISK

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The objective is to evaluate whether environmental factors, such as diet, influence serum lipid levels and metabolism and produce systemic or local alterations which are causally related to breast cancer risk. In a case - control study with controls matched for age, race and menopausal status, data on past medical and family history, dietary habits, lipid levels and cholesterol metabolism in patients with breast cancer and in controls (patients and volunteers) are analyzed. In U.S. women differences in cholesterol and in the composite risk index (CRI) were most significant in the postmenopausal group and in ethnic minorities while differences in the lipid index were significant in white women and also in the premenopausal women. In Greek women differences in CRI were more significant than cholesterol differences. Hyperlipidemia was associated with a significantly increased relative breast cancer risk. Marginal x2 test: 9.00, p less than 0.01. The findings to date suggest involvement of lipids in the disease pathway at the systemic level.

469. ROLE OF DIETARY LIPIDS IN INCREASED INCIDENCE OF MAMMARY CANCER

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Epidemiological and experimental studies have implicated dietary fat in the development of mammary tumors. To assess the possible direct effects of lipids on mammary cells, the affects of lipids on normal and neoplastic rat mammary cell growth in culture have been analyzed. Saturated fatty acids were found to inhibit while unsaturated fatty acids stimulated the growth of cells from either source. These results may be physiologically important since there is an enrichment of total unsaturated fatty acids in the glands of animals given periphenazine to stimulate proliferation. Additionally, mammary tissue has been shown to differ in the amount and types of free fatty acids released in explant culture depending on whether it contains the epithelial component or not. Less unsaturated fatty acids are released into the medium relative to saturated fatty acids when the epithelium is present in the explanted tissue. These results suggest that normal mammary epithelium recruits unsaturated fatty acids needed for growth from the surrounding adipose tissue. If this process is rate limiting for mammary cell growth then increased dietary lipids may directly supply the fatty acids to the epithelium and bypass this rate limiting process.

BIBLIOGRAPHIC REFERENCES: Monaco, M.E., Kidwell, W.R. and Lippman, M. Neurohypophysial hormone responsive cell line derived from a 7,12-dimethylbenz (alpha) anthracine induced rat mammary tumor. Biochem. J. 188: 437-441, 1980.

470. DIETARY FAT, PROLACTIN AND MAMMARY CANCER

Rogers, A. E., Massachusetts Inst. of Technology, School of Science, Dept. of Nutrition & Food Science, *77 Massachusetts Ave., Cambridge, Massachusetts,* 02139, United States of America

This study is examining the effects of long-term ingestion of high fat diets on serum hormones and on induced and spontaneous mammary tumors in rats. The relationship between composition of fats and their endocrine and tumorogenesis-enhancing effects is being studied in DMBA-treated rats fed one of several dietary fats in a purified diet. Serum hormones ae monitored by RIA, and DMBA-induced and spontaneous mammary neoplasms are identified. The possibility of predicting ultimate tumor response of animals from serum hormone profiles will be determined by comparison of values at estrus or diestrus with tumor development. The amount and distribution of prolactin and steroid receptors in mammary lesions will be determined.

Dietary fat effects on mammary gland differentiation and DNA synthesis also are being studied.

471. DIETARY FATTY ACIDS AND METABOLISM

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OBJECTIVE: Investigate the effect of dietary trans fatty acids on metabolic and physiologic parameters related to human health.

APPROACH: In specificially designed metabolic studies with experimental animals and in ancillary measurements on samples from on-going human dietary studies, the effects of dietary trans fatty acid levels on membrane structure, fatty acid metabolism related to brain development, prostaglandin metabolism, and sterol metabolism will be investigated. The occurrence and intake of trans fatty acids in the American diet will be examined with respect to the above studies, and to examine the possibility of a role of processed fats in disease states such as cancer and thrombosis.

472. LIPID LEVELS AND CHOLESTEROL METABOLISM IN RELATION TO BREAST CANCER RISK

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Thousands of women who have had cholesterol levels measured at multiphasic check-ups are being followed up to determine whether breast cancer incidence rates are related to cholesterol levels.

473. LIPIDS AND LIPOPROTEINS AS PREDICTORS OF CANCER MORTALITY

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A prospective study of cancer mortality according to lipid and lipoprotein levels at entry is being carried out. Over 5,000 subjects with over 100 cancer deaths expected during a three to five year follow-up will be included in the study, which is based on the Lipid Research Clinics Program data.

474. FAT LOSS/WEIGHT LOSS IN PATIENTS WITH CANCER

Axelrod, L., Massachusetts General Hospital, *32 Fruit St., Boston, Massachusetts,* 02114, United States of America

OBJECTIVE: Evaluate and validate the use of common metabolic techniques for the study of lipolysis, such as analysis of plasma free fatty acids, glycerol, ketones, and hormones in evaluating the nutritional status of cancer patients.

475. FAT LEVEL AND SATURATION IN CARCINOGENESIS

Troll, W., New York University, School of Medicine, Dept. of Environmental Medicine, *100 Washington Sq. E., New York, New York,* 10003, United States of America

This is part of a broader project. A summary of this subproject is not available.

476. PURIFICATION AND ANALYSIS OF ADIPOGENIC FACTOR INTERACTIONS WITH 3T3 CELLS

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The major aim of the proposed study is to lay the groundwork necessary for the elucidation of the mechanism of the adipose conversion of the 3T3 preadipocyte-fibroblast. To do this, the factor required for the conversion will be purified to homogeneity and radiolabelled. This will enable determination of (1) receptor number and dissociation constant, (2) the Ka and

concentration of factor internalized, and (3) the Km for adipose conversion. Analysis of these parameters in growing and resting preadipocytes and in mature adipocytes should yield information on how cells are programmed to respond to differentiation. The process of factor internalization will be examined, to determine if it is necessary for degradation and/or factor action. Internalization will be blocked by methylamine or cellular mutation and conversion measured. Adipose conversion in the absence of factor internalization will show that internalization is only a method of degradation.

Once this system is well characterized it should be possible to develop an assay for the event causing conversion and to isolate that molecule involved. If for example changes in the phosphorylation of nonhistone proteins occur, the kinase or phosphatase involved would be isolated and its control studied.

477. ENDOCRINE FACTORS IN CANCER-INDUCED LIPOLYSIS

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Several reports have appeared concerning the accelerated lipolysis which occurs in adipose tissue of experimental animals and humans with cancer. The general mechanisms has been shown to be due to increased fatty acid mobilization rather than to decreased basal lipogenesis. The precise mechanism underlying increased lipolysis in tumor-bearing hosts remains to be determined. Lipolysis can be influenced, both positively and negatively, by endocrine factors. The goals of the proposed research are to determine if the presence of the Walker 256 carcinosarcoma, implanted intramuscularly, in rats results in altered secretion of various lipolytic hormones as well as the antilipolytic hormone insulin. The sensitivity of the adipose tissue from tumor-bearing and control rats to many of these hormones will also be studied. The production of a lipolytic factor by the tumor cells themselves will also be studied by culturing the Walker 256 cells and testing the culture medium for lipolytic activity.

BIBLIOGRAPHIC REFERENCES: Zepp, E.A. and C.R. Reagan (1981) Plasma Insulin Levels and Adipocyte Sensitivity to Insulin in Rats Bearing Walker 256 Carcinoma. The Endocrine Society , in press.

478. MODELS OF PEROXIDIZING LIPIDS REACTIONS WITH PROTEINS AND AMINO ACIDS (RATS)

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This is part of a broader project. A summary of this subproject is not available.

479. LIPOPEROXIDATION EFFECT ON THE NUCLEUS AND CARCINOGENESIS (HUMSTER)

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This is part of a broader project. A summary of this subproject is not available.

480. NEW CLASSES OF RADIOLABELLED LIPIDS OR LIPID SEEKING AGENTS

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This is part of a broader project. A summary of this subproject is not available.

VII. BIOCHEMICAL AND BIOPHYSICAL STUDIES OF THE CELL MEMBRANE AND TRANSPORT MECHANISMS

A. MEMBRANE BIOSYNTHESIS AND MEMBRANE DYNAMICS

481. BIOGENESIS OF MEMBRANE

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In the past eight years, supported by a grant from the American Cancer Society, we have established an ideal model system which enables us to investigate the biogenesis of the membrane from many different aspects. This system consists of a major structural membrane protein from the membrane of Escherichia coli (the lipoprotein of 7,200 molecular weight). Especially in the past two years we have achieved quite a few breakthroughs including the determination of the complete amino acid sequence of the prolipoprotein, a precursor of the lipoprotein, and the determination of the complete nucleotide sequence of this lipoprotein mRNA, and the complete nucleotide sequence of . DNA fragment of about 700 bases containing the lipoprotein g ne. Furthermore, we have shown that assemble intermediates of membrane components are accumulated by reducing the fluidity of membrane lipids.

In the present application, on the basis of the above results we will further promote our efforts on (1) genetic manipulation of membrane biogenesis with the use of clones carrying genes for membrane proteins, (2) further characterization of precursors and assembly intermediates accumulated by reducing the fluidity of membrane lipids, and (3) examination of the possible existence of a cytoplasmic membrane protein(s) which may be required for the secretion of proteins across the cytoplasmic membrane.

482. CHARACTERIZATION OF MOLECULAR COMPONENTS INVOLVED IN MEMBRANE FUSION

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Exocytosis of secretory proteins and plasma membrane growth are both the end products of complex pathways brought about by a series of molecular events involving lipids, proteins and glycosylated peptides. The coordination of this pathway, the movement of the vesicle to the terminal membrane, the juxtaposition of the two membrane systems, recognition of fusion sites and the actual fusion event are postulated but uncharacterized processes.

The aim of this research project is to purify two membrane structures from S. cerevisiae: the secretory vesicles and the plasmalemma. This will allow the characterization of their composition and an analysis of the molecules which direct the specificity of their association. These studies will be coupled with the analysis of the same structures from mutants blocked in the secretion process. The eventual goal is the elucidation of the pathways of membrane assembly and protein secretion, understanding in detail the molecular mechanisms by which each step occurs.

483. MEMBRANE MUTANTS OF MAMMALIAN CELLS

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The overall aim of this project is to use a combined genetic and biochemical approach to studies of the structure, function and biosynthesis of the plasma membrane of animal cells. In previous studies we have shown that lectins may be used to select cell lines which possess specific alterations in certain carbohydrate structures at the cell surface. One of these mutants lacks a particular glycosyltransferase enzyme activity and investigations of this mutant have shed light on mechanisms of glycoprotein biosynthesis in mammalian cells, on structural aspects of the CHO cell surface, and on functional aspects of the carbohydrate moieties of glycoproteins. We have selected a further

seven, genetically-distinct lectin-resistant (LecR) mutants from the Chinese hamster ovary (CHO) cell line. Five of these have been shown to possess specific defects in cell surface carbohydrate but none has so far been shown to lack any particular enzyme activity. In future studies, we will continue to search for the molecular bases of lectin-resistance in each LecR cell line. Also, we hope to isolate new CHO membrane mutants using schemes designed to select for temperature-sensitive, conditional-lethal cell lines. This approach may give rise to cells which possess a normal membrane at one temperature but a non-functional membrane at another temperature and thus provide a biological system for examining criteria of membrane function.

484. ORIGIN AND RELATIONSHIPS OF CELLULAR MEMBRANES

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The general aim of this research is to study the origin, development and relations of membranous cytoplasmic organelles and to determine their relation to the nucleus. Our approach makes use of microsurgery on single cells combined with electron microscopy and related techniques, including electron microscope cytochemistry and radioautography. The study consists of the following parts. First, the formation of the Golgi apparatus can be brought about by transplanting a nucleus into enucleate amoebae. We propose to determine the ability of the nucleus at different stages of the cell cycle to promote the development of the golgi apparatus and other cytoplasmic organelles. Second, when amoebae are injected with heterologous cytoplasm, foreign organelles are segregated and digested within vacuole-like structures in the host cytoplasm. We propose to determine some of the metabolic processes involved in this response by use of various inhibitors. Third, we plan radioautographic studies of the synthesis, transport and turnover of cell surface material and on the relation between intracellular organelles and the surface. Enphasis will be placed in identifying factors that affect or regulate surface production and turnover, and determining how these processes respond to different experimental conditions. Finally, the relation between the nuclear envelope and cytoplasmic membranes will be determined using microinjection of precursors, transplantation of nuclei with radioactively labeled nuclear membranes, and electron microscope radioautography.

485. BIOPHYSICAL STUDIES OF TISSUE FUNCTION AND CELLULAR STRUCTURE ASSOCIATED WITH MALIGNANCY

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OBJECTIVE: To study molecular motion and structural change in cell membranes and protoplasm.

APPROACH: Electron spin resonance spectroscopy (ESR) is used to examine the environment and motion of spin labels such as N-oxyl-4', 4'-dimethyloxazolidine derivative of 5-ketostearic acid (5-doxyl stearic acid) in membranes and 2,2,6,6: tetramethyl 4-amino piperidine 1-oxyl (tempamine) in protoplasm.

PROGRESS: Spin label studies of cell membranes of Ts14 monkey epithelial cells grown at 33 degress C and 39.5 degrees C (restricted temperature) reveal changes in the membrane. These are demonstrated in the parameters 2T11 and 2T1 usually measured in spin label spectra of this type and also in the shape of the spectra. These changes may indicate differences in fluidity ordering or molecular motion of the spin label in membrane. Computer simulation of the spectra should give more information. Slight differences in the spin label spectra obtained from 2 lines of Friend cells only one of which can be stimulated to mature by treatment with dimethyl sulphoxide is also suggested.

It is thought that spin labelled phospholipids may give more specific information in certain cases. Since these are not commercially available, the synthesis and purification of these labels has been carried out, with the helpful advice of Dr. P. Knowles, University of Leeds.

In the studies using tempamine it is necessay to quench the signal from extracellular spin label. Potassium ferricyanide has been used for this purpose. However, experiments have shown that, under certain conditions, the spectrum from the tempamine in erythrocytes can be modified by the presence of ferricyanide. There is also evidence that the ferricyanide affects the cell membrane. The use of potassium chromium oxalate as a broadening agent is now being studied.

486. BIOCHEMICAL AND IMMUNOLOGICAL STUDIES ON CELL SURFACES AND PLASMA MEMBRANES

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OBJECTIVE: Describe the chemical and physical properties of a select group of membrane proteins and determine their organization and interaction within the membrane. Isolate individual protein and glycoprotein components of the milk fat globule and characterize them chemically and immunologically. Isolate the two major plasma membrane glycoproteins of the ascite cells and characterize them chemically, physically, and immunologically.

APPROACH: Isolate and characterize particular, important membrane components, study their organization and interactions by chemical, enzymatic and immunological localization procedures and investigate their roles in cellular activities by chemical, immunological and enzymatic perturbation experiments.

PROGRESS: The cell surface properties of the 13762 rat mammary adenocarcinoma are being studied in an effort to understand relationships between morphology, transplantability, receptor mobility and sialoglycoproteins. Comparisons between a xenotransplantable (MAT-C1) and non-xenotransplantable subline (MAT-B1) have been most informative. MAT-C1 cells have numerous highly branched microvilli extending from the cell body, restricted mobility and shedding of cell surface Concanavalin A (Con A) receptors and a 5-fold greater content of sialic acid. MAT-B1 cells have fewer microvilli, which are unbranched, and greater mobility and shedding of Con A receptors. The branched microvilli can be induced to shed from MAT-C1 cells by hypotonic swelling and Con A treatment. They contain extensive amounts of actin and a major cell surface cialoglycoprotein (ASGP-1). ASGP-1 can be observed on cell surfaces of both MAT-B1 and MAT-C1 cells by chemical, enzymatic and histochemical labeling. By metabolic labeling with glucosamine, it is the predominant glycoprotein synthesized by both sublines. The glycoproteins have been isolated from cell surface membranes of these cells by a one-step CsCl density gradient centrifugation procedure. MAT-C1 ASGP-1 is larger than MAT-B1 ASGP-1 by ultracentifugation or gel filtration and contains more carbohydrate, particularly sialic acid, fucose and galactose. However, the amino acid compositions of the two are essentially identical.

487. DYNAMICS OF BIOPHYSICAL MEMBRANE PROCESSES

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The proposed research continues investigations of the dynamics of molecular processes which occur on the surface membranes of animal cells. Its purpose is to understand the molecular mechanisms of control of growth, differentiation and function of normal and cancer cells. Measurements by fluorescence photobleaching recovery of lateral molecular motion by diffusion and cooperative driven flow of fluorescence labeled proteins and lipid analogs are emphasized. Of particular concern is regulation of mobility by coupling with the cytoskeleton. Current projects include (1) measurements of the restriction by the cytoskeleton of receptor and antigen mobility on lymphocytes and acetylcholine receptor on myotubes, (2) mobility and clustering of the receptor for low density lipoprotein, (3) modulation of mobility by Con A platelets, (4) assembly of viruses in and on cells and (5) effects of cholesterol on protein mobility. New techniques for topographical analysis of motility and cooperative motion of fluorescence labeled molecules on the cell surface using video image acquisition and analysis are being developed and applied.

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488. ANALYSIS OF EUCARYOTIC CELL SURFACES

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The hypothesis being tested is that the initiation and maintenance of growth control is a function of intercellular adhesion, probably affected by macromolecular events at the level of the cell membrane. These phenomena must involve the interaction of specific molecules that trigger further changes on the surface of the participating cells. Since transformed cells have a lower level of tissue organization and altered growth and adhesive properties, these differences would be reflected in altered membrane architecture and/or molecular fine structure. Transformed and normal cells show such differences with respect to complex carbohydrates (glycoproteins and glycolipids). In some instances complex carbohydrates have been implicated as playing a role in growth regulation and in intercellular adheshion which ultimately governs morphogenesis and metastasis.

The proposed research plan is a direct approach to isolate compounds from the surfaces and members of synchronized cells that have the ability to influence growth and adhesion, and to determine the biochemical event involved in those interactions.

BIBLIOGRAPHIC REFERENCES: Procollagens Rich in 3-Hydroxyproline. C.E. Schwartz, C.G. Hellerqvist and L.W. Cunningham, Biochim. Biophys. Res. Comm. 90 240 (1979). Intercellular Adhesion as a Function of the Cell Cycle Traverse. C.G. Hellerqvist, J. Cell Biol. 82 682 (1979).

489. LATERAL MOBILITY OF MOLECULES AT CELL SURFACE

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The major objective of this proposal is to measure the translational mobility of various cell surface components in single, living cells using a laser excited fluorescence microscope. This will be accomplished by labelling the cell fluorescent molecules specific for the cell surface and either measuring the fluorescence recovery after photobleaching in small areas of the cell surface or electronically observing spatial variations with time in the fluorescent image of the entire cell. Measurement of the mobility of plasma membrane components will be made when cultured cells are treated with various pharmacological agents, multivalent ligands to cell surface receptors, and growth factors and as a function of phase in the cell cycle and cell density and whether or not the cell has been neoplastically transformed. These measurements will assist in the development of a more refined understanding of the cell surface and the relationship of cell surface dynamics to the physiological situation of the cell. Accumulating evidence indicates that changes in the mobility of membrane components and membrane 'fluidity,' probably manifest in alterations in membrane transport, enzyme activity and cellular recognition phenomena, may play an important role in the development of certain diseases, particularly the neoplastic transformation.

490. THE PLASMA MEMBRANE IN NORMAL AND CANCER CELLS

Racker, E., Edelstein, S., Hammes, G., Heppel, L., Hinkle, P., Podleski, T., Utermohlen, V., Webb, W., Hess, G., Cornell University, Ithaca Campus, School of Arts & Sciences, Dept. of Biological Sciences, *Ithaca, New York*, 14850, United States of America

This program grant aims to coordinate the efforts of several investigators on the Cornell Campus in their studies on membranes of normal and cancer cells. The greatest emphasis will be place on the plasma membrane and the following parameters will be studied. 1) The mechanism of lactic acid excretion in normal and cancer cells. 2) The mobility of phospholipids and proteins in the plasma membrane of normal and cancer cells. 3) Permeability changes due to phosphorylation of transformed cells by external ATP. 4) Receptor-controlled ion fluxes in normal and cancer cells. 5) Characterization of the glucose transporter of the plasma membrane. 6) Molecular interactions between cells and lectins and hormones. 7) Structure of the plasma membrane and membrane components as studied by electron microscopy.

491. MOTILITY-COUPLED CELL SURFACE RECEPTORS

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Eukaryotic cell surfaces are extremely dynamic structures. Important questions concerning cell surface dynamics include: 1) The mechanism by which cells rapidly change their surface properties, 2) The nature of motility-coupled cell surface receptors, 3) The mechanism of plasma membrane-associated force transduction, and 4) The nature of cell surface-cytoskeleton linkages. A number of diseases (including cancer, familial hypercholesterolemia, and certain forms of diabetes) involve an alteration in the nature or distribution of cell surface receptors. The Chlamydomonas flagellar surface exhibits many dynamic cell surface properties and has certain unique technical advantages as an experimental system for studying problems of cell surface dynamics, in part because the flagellar membrane represents a functionally unique subset of the cell's plasma membrane possessing a limited complement of proteins and glycoproteins. A combination of biochemical, genetic, immunological and ultrastructural approaches will be utilized to 1) Study protein turnover in the flagellar membrane, 2) Identify and characterize the motility-coupled flagellar surface receptor, 3) Identify the components of the motor responsible for flagellar surface motility, 4) Characterize the components responsible for membrane-axonemal linkage, 5) Identify ultrastructural changes accompanying changes in flagellar surface properties, and 6) Isolate and characterize mutant strains of Chlamydomonas defective in flagellar surface motility and possessing alterations in the principal flagellar membrane glycoprotein.

492. PROTEIN-LIPID INTERACTION IN BIOLOGICAL MEMBRANES

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The understanding of the principles involved in membrane protein-lipid interaction is essential for the analysis of most membrane phenomena, including the well-known alterations of membranes in cancer cells. It is proposed to study the protein-lipid interaction by using a simple biological membrane, the outer membrane of E. coli, as a model. We shall purify, and suitably label with spin labels, (1) the protein porin which constitutes nearly 50% of all the proteins in this membrane, and (2) two kinds of lipids present in this membrane, lipopolysaccharide and phospholipids. We shall first test whether specific lipid species are necessary for the proper functioning of the protein, by testing for the function of the protein (opening of passive diffusion pores) in protein-lipid mixtures. We shall then try to locate the sites of interaction in the participating molecules by the use of spin-labeled components. Finally, we shall test the role of protein-lipid interaction in the asymmetric structure of the biological membranes by reconstituting the membrane under the condition that all porin molecules are facing in the one direction.

493. SPIN LABELING AND MEMBRANE STRUCTURE

Griffith, O. H., Jost, P. C., Birrell, G. B., Silvius, J. R., Volwerk, J. J., Oregon State Higher Education System, University of Oregon, Inst. of Molecular Biology, *Johnson Hall, Eugene, Oregon*, 97403, United States of America

The central theme of this project is a study of lipid-protein interactions in biological membranes with the eventual aim of understanding the molecular details of lipid-protein associations. The strategy is to introduce spin label analogues of specific, naturally occurring membrane phospholipids into preparations containing a single kind of functional protein, and to study lipid mobility and distribution by electron spin resonance.

Lipid specificity, involving one or more sites in a background of non-selective sites on the hydrophobic surface of transmembranous proteins, has been demonstrated for the Na,K-ATPase using both single-tail and phospholipid spin labels. Our current objectives include characterization of specific lipid binding using ESR data, DSC data, electron microscopy and activity assays. We are planning to initiate work on the potential specificity of lipid-protein interactions in the association of phospholipase A2 with lipid interfaces.

BIBLIOGRAPHIC REFERENCES: Jost, P.C. and Griffith, O.H. (1980). The Lipid-Protein Interface in Biological Membranes, Ann. N.Y. Acad. Sci. 348, 391-407. Jost, P.C. and Griffith, O.H. (1980). Lipid-Lipid and Lipid-Protein Interactions in Membranes, Pharmacology Biochem. and Behavior 13, 155-165.

494. MEMBRANE PROTEIN-PHOSPHOLIPID INTERACTIONS

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The evidence for proteins being important components of biological membranes and for their implications in membrane structure and function is of considerable interest. The integral, or intrinsic, protein components, intercalated into the bilayer which by definition are tightly bound to the membrane, are in many cases amphipathic. The suggestion that the hydrophobic sections of these membrane bound proteins serve to anchor the proteins to the membrane by virtue of hydrophobic interactions between these regions and lipids has certainly received much attention recently.

The aims of the proposed work are to investigate the regions of several intrinsic membrane proteins (glycophorin A from the erythrocyte membrane, cytochrome b5 from liver, and NADH-cytochrome b5 reductase from liver) which have been suggested to interact with lipids. The general approach will involve reconstituting proteins in lipid systems composed of lipids with covalently attached photosensitive groups, the locations of which can be synthetically varied along the fatty acyl chains. Photolysis and subsequent analysis should reveal regions of contact between a particular carbon atom of the fatty acid chain and particular amino acid residues.

495. MEMBRANE CONTROL OF MICROSOMAL CYTOCHROME P-450

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The hepatic monooxygenase system consisting of NADPH-cytochrome P-450 reductase and cytochrone P-450 is involved in the oxidative metabolism of xenobiotics including therapeutic drugs, carcinogens, and environmental pollutants such as industrial wastes and pesticides. This enzyme system is also in the metabolism of endogenous compounds such as steroids and fatty acids. Both enzymes are intimately associated with the microsomal membrane and this forms the basis upon which this proposal is founded. From previous studies of this system as well as studies of other membrane bound enzymes, it appears that the lipid components of membranes influence the properties of membrane bound proteins. The objective of the studies outlined in this proposal is the elucidation of lipid-protein and protein-protein interactions within the microsomal membrane which influence the substrate specificity and the rate of dehydroxylation catalyzed by the cytochrome P-450 system. To achieve this objective, experiments are outlined which involve the systematic in vitro modification of the lipid composition of the microsomal membrane and subsequent investigation of alterations in substrate binding and kinetic parameters. Microsomal fractions isolated from untreated rats as well as from rats pretreated with phenobaribtal, 3-methylcholanthrene, and pregnenolone-16 alpha-carbonitrile will be used. Several techniques not previously utilized for the study of the hepatic monoxygenase system will be employed including phospholipid substitution of native and organic solvent extracted microsomal membranes. Phospholipid substitution will be achieved through the use of detergent or phospholipid exchange proteins. These studies should yield results which will be applicable to other membrane bound enzymes in addition to the hepatic monooxygenase system.

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B. TRANSPORT OF NUTRIENTS INTO NORMAL AND NEOPLASTIC CELLS

496. REGULATION OF MEMBRANE TRANSPORT SYSTEMS AND MEMBRANE TURNOVER IN CARCINOGENESIS

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OBJECTIVES: 1. To explore the physiological mechanisms by which cells regulate the major driving forces to which growth associated transport activities are linked. 2. To further our understanding of the means by which the activities of transport systems are enhanced upon transformation.

MAJOR FINDINGS: The contractor's operating hypothesis is that one of the early effects of the tumor promoter, TPA (tetradecanoyl phorbol acetate), on sensitive cells is an enhancement of membrane potential. A consequence of this change is an enhanced driving force on potential-coupled amino acid uptake and the increased availability of these protein precursors required for cell growth. Using the amino acid analog AIB (amino isobutyric acid) and Friend erythroleukemic cells, it was shown that the expected potential change is detectable 30 minutes after treatment of the cells with TPA and is complete within 2 hours; the change does not occur in cycloheximide treated cells and thus is dependent on the synthesis of new protein. All of the enhancement may be abolished by treatment of the cells with cardiac glycosides that block alkali cation transport, suggesting that the increased potential is due to an uncoupling of the Na ion -K ion transporter and the development of electrogenicity by this electrolyte transport system.

The effects of TPA appear to be associated with an obligatory minimum level of free cytosolic Ca ions. TPA is known to stimulate the induction of ornithine decarboxylase in quiescent mouse 3T3 cells, a specific response that was shown to be abolished if the cellular Ca ions is depleted with the use of the Ca ions inophore A23187. The levels of cell Ca ions are now being backtitrated to determine the minimum levels required for the induction to occur.

A new model system is being developed to study the effects of TPA and other promoters and/or carcinogens on differentiating epithelial tissues in vitro with special emphasis on their transport properties. The cells being used are a pig kidney line, LLC-PK1, which have a fibroblast-like morphology during log growth but which, on confluence, take on classical epithelial NaCl-transport functions. These cells also develop Na ion-dependent hexose transport characteristic of differentiated kidney proximal tubule epithelia. (Text Abridged.)

497. NMR STUDIES OF BIOLOGICAL MEMBRANES

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The main purpose of this proposed research is to understand the structure-function relationships in biological membranes, in particular the relationship between membrane transport and its molecular structure. We have chosen bacterial membranes as models for biological membranes. Our basic experimental approach is to combine chemical, genetic, and physical techniques to investigate membrane transport. Chemical techniques are needed to synthesize a number of fluorinated and deuterated fatty acid and amino acid analogs to be biosynthetically incorporated into phospholipids and membrane proteins as NMR probes. Genetic techniques are needed to isolate appropriate auxotrophs for the incorporation of these NMR probes into phospholipids and binding proteins and to isolate suitable mutants for reconstituting membranes from components. 1H, 2H, and 19F NMR techniques will be used to detect conformational changes in the fluorine and deuterium labeled binding proteins and phospholipids in membranes during the transport process.

We shall study the properties of the binding proteins, phospholipids, model membranes, and reconstituted membranes in relation to membrane transport. It is expected that one of the important developments in biomedical sciences during the next few years will center on explaining the roles of membranes in living cells and on explaining the structure-function relationships of 'altered' membranes in malignant cells (such as cancer cells). We hope that the results obtained from this proposed research will contribute to an understanding of some of the essential cellular processes not only in bacterial cells but also in mammalian cells.

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498. STUDIES OF NORMAL AND MALIGNANT CELL MEMBRANES

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The relationships between physiological functions of membranes and the relevant molecular structures will be studied, with special emphasis on the plasma membrane. Major goals are to define the molecular mechanisms responsible for cell-cell recognition and adhesion, solute transport, and the interactions between the plasma membrane and certain hormones and toxic substances. Exploratory experiments will also be conducted on related phenomena, such as the regulation of adenylate cyclase by certain solute transport systems, and the potential role of cell surface carbohydrates in growth control and motility. Many of the proposed studies will use liver cell membranes isolated for normal embryonic and fully differentiated hepatocytes, hepatomas, and regenerating liver cells. Other eukaryotic cells will be used, including adipocytes, intestinal mucosa, and normal and transformed tissue culture cell lines. The glucose transport systems of enteric bacteria will be examined in detail. These systems offer a rare opportunity for reconstructing functional units of the plasma membrane from their homogeneous components; the results should provide a precise molecular description of the structure-function relationships of this class of membrane proteins, and how they interact with cytoplasmic and other membrane proteins (adenylate cyclase, other solute transport systems). The structural studies will also provide the necessary background for future structure-function experiments to be conducted with plasma membrane components involved in adhesion, hormone and toxin binding, etc., following their isolation and identification.

The wide variety of techniques to be applied to the biological systems include the use of fluorescent and electron spin resonance probes, and the results will be correlated with hydrodynamic, equilibrium and kinetic measurements on the same systems.

The proposed work should yield information on the molecular mechanisms responsible for these physiological functions of the plasma membrane, and how and whether they change in malignancy, metastasis, and in normal embryonic development.

499. STRUCTURE AND FUNCTION OF B12-BINDING PROTEINS

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My research objective is to elucidate the mechanism by which vitamin B12 transport proteins facilitate the cellular uptake of vitamin B12.

Plasma transcobalamin II facilitates the uptake of vitamin B12 by a number of different cells. We have isolated human and rabbit transcobalamin II in homogeneous form and have developed methods for labeling these proteins with radioactive iodine. These labeled proteins will be used to study the role and fate of transcobalamin II during the complex process in which vitamin B12 binds to cell surfaces, enters cells, migrates to various sub-cellular fractions, and exits from cells. 58Co-vitamin B12 and a variety of 57Co-vitamin B12 analogues will be employed to study the importance of, and alterations which occur in, the structure of vitamin B12 during these processes.

Human R-type vitamin B12-binding proteins with low sialic acid contents transport vitamin B12 exclusively to the liver. 125I-labeled R-type proteins, 58Co-vitamin B12, and 57Co-vitamin B12 analogues will be employed to elucidate this process and to determine its biological significance. We also plan to characterize the process by which structurally abnormal R-type proteins are synthesized in chronic myelogenous leukemia and other myeloproliferative diseases.

Intrinsic factor facilitates the uptake of vitamin B12 by ileal mucosal cells. We plan to isolate and characterize the ileal cell surface receptor for the intrinsic factor-vitamin B12 complex. We plan to label intrinsic factor in vivo with 3H-amino acids and utilize this radioactive protein to elucidate the mechanism by which intrinsic factor facilitates the ileal absorption of vitamin B12.

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500. CELLULAR INCORPORATION OF VITAMIN B-12

Gams, R. A., Ostroy, F., University of Alabama in Birmingham, School of Medicine, Dept. of Medicine, *1919 7th Ave. S., Birmingham, Alabama*, 35233, United States of America

The purpose of this investigation is to define the mechanism by which vitamin B-12 is incorporated by mammalian cells. In vitro experiments will be performed with isolated rat ileal and hepatic cells, murine leukemia cells, and hamster embryo cells both before and after neoplastic transformation. Utilizing purified B-12 transport proteins, we plan to look at B-12 incorporation and processing by whole cells and isolated subcellular compounds. We wish to examine similarities and/or differences in uptake mediated by intrinsic factor, transcobalamin II and R-binders of B-12 in normal and neoplastic cells to further our understanding of B-12 transport and possibly to help define the role of B-12 in mammalian cellular metabolic functions.

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501. NONELECTROLYTE TRANSPORT IN RENAL TUBULES

Schafer, J. A., Barfuss, D. W., Heinz, A., University of Alabama in Birmingham, School of Medicine, Dept. of Physiology & Biophysics/Medicine, *1919 7th Ave. S., Birmingham, Alabama*, 35233, United States of America

This project is designed to provide information on the general characteristics of nonelectrolyte transport in the mammalian proximal tubule and in the Ehrlich ascites tumor cell. The methodology for renal studies will involve the perfusion of isolated tubule segments including the proximal convoluted tubule, and early and late segments of the proximal straight tubule. Specifically, our aims are: 1) To determine the active sites for amino acid and sugar absorption by the proximal tubule. 2) To describe the mutual dependence of Na ion and nonelectrolyte absorption. 3) To describe the potential regulatory role of nonelectrolyte absorption on salt and water absorption in the proximal nephron. 4) To determine the nature of the nonelectrolyte backflux from peritubular medium to the lumen in proximal tubules, and to describe the potential physiologic and pathologic modifiers of this backflux pathway. 5) To examine the possibility that sugars and amino acids may be actively absorbed in other nephron segments. 6) To develop mathematical models for the interrelationship between nonelectrolyte, salt and water transport. 7) To describe the mechanisms whereby cellular energy is coupled to the active accumulation of amino acids in the Ehrlich ascites tumor cell.

502. ONCOGENY AS BLOCKED ONTOGENY - STUDIES OF LIVER CANCER

Potter, V. R., Kelley, D., Benjamin, H., Campbell, H. A., Becker, J. E., Brania, H., University of Wisconsin, Madison Campus, Medical School, Dept. of Oncology, *450 N. Randall Ave., Madison, Wisconsin,* 53706, United States of America

Project is carried out at two levels, in vivo and in vitro.

In vivo studies include a long-range program of chemical carcinogenesis using a modified Peraino protocol. Acetyl aminofluorene (AAF) was fed to weanling male and female rats for 2 weeks only, at three levels - 0.015, 0.030, and 0.045% - in semipurified diet containing 30% casein. Tumor incidence, enzyme altered foci, and thymidine autoradiographs noted at various times in groups receiving 0.05% phenobarbital or basal diet, or partial hepatectomy at the age of 28 days during AAF feeding; total number of groups equals 29. (Still in progress).

In vitro studies are focussed on primary liver cell cultures from rats at various ages, fasting and fed, or following partial hepatectomy. Four hepatoma cell lines in serial culture are used in parallel studies. Measurements emphasize amino acid and K-ion transport with various media and hormone combinations. A computer program has been utilized to quantitate the respective contributions of high Km and low Km systems based on initial velocities of uptake at varying substrate concentrations. Other measurements include assays for cyclic AMP, tyrosine aminotransferase and ornithine decarboxylase. The entire complement of newly synthesized soluble proteins labeled in culture with 35S-methionine with no hormone and hormone combinations is separated by 2-dimensional electrophoresis, and a computer program has been developed to quantitate the radioactivity of 101 individual spots based on autoradiography and microdensitometry.

BIBLIOGRAPHIC REFERENCES: Kelley, D. S. and Potter, V. R. Regulation of Amino Acid Transport Systems by Amino Acid Depletion and Supplementation in Monolayer Cultures of Rat Hepatocytes. J. Biol. Chem. Dec. 25, 1978 253: 9009-9017 (1978). Kelley, D. S. and Potter, V. R. Repression, Derepression, Transinhibition, and Trans-stimulation of Amino Acid Transport in Rat Hepatocytes and Four Rat Hepatoma Cell Lines in Culture. J. Biol. Chem. 254: 6691-6697, 1979.

503. TUMOR ASSOCIATED CHANGES OF INTESTINAL AND LIVER CELLS

Isselbacher, K. J., Herscovics, A. A., Alpert, E., Quaroni, A., Harvard University, Boston Campus, Massachusetts General Hospital, Gastrointestinal Unit, *32 Fruit St., Boston, Massachusetts,* 02114, United States of America

The objectives of this Program Project are aimed at studying the various aspects of the behavior and characteristics of normal and malignant cells, with a special emphasis on cells of the intestinal tract and liver. Major areas of investigation will include the following: (1) Alterations in transport of nutrients, especially sugars and amino acids, between normal and malignant fibroblasts and epithelial cells. Major emphasis will be placed on surface membrane changes in relation to transport and the technique of measuring translocation across membrane vesicles and reconstituted vesicles (i.e., liposomes) will be used. (2) We will continue to assess the clinical significance of a tumor-associated isoenzyme of galactosyltransferase activity found in the sera and tumors of animals and patients with malignant neoplasms. This isoenzyme and a recently detected inhibitor of tumor growth found in animals with large tumors will be studied for their effects on cell growth in tissue culture systems and animal tumor models. (3) In addition, an in-depth investigation into the synthesis of surface glycoproteins and the role of lipid intermediates in glycoprotein synthesis, especially of intestinal cells, will be carried out. (4) We plan to purify a tumor associated alpha-H2globulin, a recently described oncofetal protein, and tumor associated isoferritins, from liver and intestinal tract tumors. We plan to develop antisera to these tumor associated proteins which may be of diagnostic significance.

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504. REGULATION OF AMINO ACID TRANSPORT IN FIBROBLASTS BY CELL-CELL INTERACTIONS

Lieberman, M. A., Harvard University, Boston Campus, School of Public Health, Dept. of Nutrition, *55 Shattuck St., Boston, Massachusetts,* 02115, United States of America

Transformed cells do not exhibit the regulation of amino acid transport observed in normal cells. In an effort to understand the mechanism responsible for this loss of regulation in transformed cells, the regulation of amino acid transport by cultured mammalian cells will be investigated. Particular emphasis will be placed on the role of cell-cell interactions using the established mouse cell line, Swiss 3T3. The cell surface associated factors which regulate the sodium dependent amino acid transport system (A system) will be defined, solubilized, and isolated. Once isolated, a study of the mechanism of action of this factor, along with a biochemical characterization and an examination of the in vitro effects of the factor on growing cell cultures, will be undertaken. In addition, the relationship of this factor to other growth control related factors, with respect to cellular expression as a function of the cell cycle and culture density, will be studied. Similarly, the regulation of the expression and/or activity of these regulatory factors will be examined with particular emphasis on covalent modifications, especially phosphorylation.

505. REGULATION OF AMINO ACID TRANSPORT IN FIBROBLASTS BY CELL-CELL INTERACTIONS

Lieberman, M. A., Harvard University, Boston Campus, School of Public Health, Dept. of Nutrition, *55 Shattuck St., Boston, Massachusetts,* 02115, United States of America

No summary has been provided to the Smithsonian Science Information Exchange.

506. SEARCH FOR CHARACTERIZATION OF METABOLIC CHANGES CAUSED BY MALIGNANCY (INCREASE IN FACTORS INVOLVED IN THE GLUCOSE TRANSPORT SYSTEM)

Lipmann, F., Heden, L. O., Rockefeller University, Biosynthesis Lab., *66th & York Ave., New York, New York,* 10021, United States of America

The study of the glucose transport system will be continued. Much emphasis will be placed on the separation of glucose binding and transport factors and on characterization of the difference between normal and malignant tissue membranes with regard to the much stronger binding of the system in the latter. We hope to be able to check the recently reported claims of glucose binding activity found in the malignantly connected altered glycoproteins of the membrane surface. If true, this would support a qualitative connection of the ubiquitous quantitative increase of glucose transport with transformation. We also aim to compare the antigenicity of the transport factors(s) from normal and transformed cell membranes. We would like to extend our investigations of the above-mentioned, so far insufficiently studied, separability of glucose binding and transport by attempting to separate them from a chromatographically overlapping peak by use of a variety of elution media. In particular, a thus obtained separate binding factor may help the often observed change from reversible to irreversible glucose binding. The chemical characteristics of glucose bound in the two states could then be studied.

507. EARLY CELLULAR CHANGES IN VIRAL ONCOGENESIS

Weber, M. J., University of Illinois, Urbana Champaign Campus, School of Life Sciences, Dept. of Microbiology, *Urbana, Illinois,* 61801, United States of America

Our overall objectives continue to be to increase understanding of the cellular and molecular events responsible for malignant transformation by Rous sarcoma virus.

For the current year, we are concentrating on two areas: identification and characterization of the glucose transporter from chicken embryo cells transformed by RSV; and exploitation of 'partial' transformation mutants of RSV to analyze the functional significance of RSV-induced phosphorylations.

508. REGULATION OF TRANSPORT IN CULTURED ANIMAL CELLS

Kalckar, H. M., Boston University, School of Arts & Sciences, Dept. of Chemistry/Biology, *881 Commonwealth Ave., Boston, Massachusetts*, 02215, United States of America

Oncogenic transformation of animal cell cultures and sugar deprivation result in markedly enhanced nutrient transport or uptake. How rates of transport are controlled is not known and how increased nutrient uptake relates to neoplastic potential has not been determined. In this proposal efforts to clarify these relationships focus on uptake regulation and utilize two different cell culture systems: 1) regulation of uptake by non-tumorigenic and tumorigenic cells by manipulation of sugar concentrations in culture media and 2) uptake and transport regulation in a highly neoplastic line which has low hexose uptake and high amino acid transport. In the initial approach, separation of the general process of nutrient uptake into nutrient transport and nutrient metabolism is outlined. Using whole cells, emphasis is placed on the uptake of 2-deoxy-D-glucose and D-galactose as models for hexose uptake systems and on the transport of cycloleucine as a model amino acid transport system. Inhibitors of protein synthesis (cycloheximide and puromycin) and hexose transport (cytochalasin B) are considered for use in studies of how protein turnover might control uptake systems. Plans for the use of these inhibitors in promoting large differences in uptake rates are also outlined. Efforts to label specific transport molecules are likewise planned based on large fluctuations in transport rates promoted by selective inhibition and recovery from inhibition. Regulation of amino acid transport systems (using analogues such as alpha-amino isobutyric acid) will also be examined, since glucose starvation, as well as oncogenic transformation, seems to 'induce' derepression also in this system. Studies of transport of sugars by plasma membrane vesicles derived from transformed and untransformed cells are planned. A scheme for carrier purification (principally for the galactose transport molecule) and methods for the detection (assay) of the carrier are described. Carrier mediated transport and subsequent metabolism of nutrients are the focus of the study relating to the highly tumorigenic line of BHK cells. This section attempts to correlate the degree of anomalous nutrient uptake with in situ neoplastic potential. Thus the focus of the effort is to get a more clear characterization of how a readily measured carrier system is regulated. (Text Abridged.)

509. DRIVING FORCES FOR NUTRIENT TRANSPORT IN TUMOR CELLS

Smith, T. C., University of Texas, Health Science Center at San Antonio, Graduate School of Biomedical Sciences, Dept. of Physiology, *7703 Floyd Curl Dr., San Antonio, Texas*, 78284, United States of America

The 'Na ion gradient hypothesis' proposes that in animal cells the electrochemical potential gradient for Na ion, which is established by active Na/K transport, serves as the sole energy source for driving accumulation of certain organic solutes by the cell. The major controversy about this postulate is the adequacy of the energy available from the cation gradients to totally energize amino acid uptake.

This proposal is based on the hypothesis that the cell membrane potential plays an essential role in both the regulation of cellular electrolyte activity and the provision of nutrients necessary for cellular metabolism. The long-range goal of the project is to characterize the role of the membrane potential as an important component of nutrient uptake, and in particular amino acid transport, essentially for maintenance of cell integrity and growth. To accomplish this objective we will investigate in Ehrlich ascites tumor cells: (1) the effect of amino acid transport on the cell membrane potential; (2) the effects of amino acids on the intracellular Na ion and K ion electrochemical activity; (3) the coupling stoichiometry of cation-amino acid co-transport; and (4) how experimental alterations of the membrane potential affect amino acid transport. Membrane potential and cytoplasmic cation activities will be measured with intracellular, glass microelectrodes.

We anticippte that this investigation will define the role of the cell membrane potential in the regulation of amino acid movements and lead to a more complete understanding of the energetics involved in meeting the cell's nutritional requirements.

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510. MEMBRANE DYNAMICS IN NORMAL AND TUMOR CELLS

Quinlan, D. C., West Virginia University, School of Arts & Sciences, Dept. of Biology, *Morgantown, West Virginia*, 26506, United States of America

Benzo-pyrene-transformed 3T3 (BP-3T3) cells can be arrested in the G1-phase of their cell cycle by a 100-fold reduction in the serum concentration of the growth medium. Under such conditions alpha-aminoiso-butyric acid uptake is reduced 3-fold. However, the uptake of uridine and certain glucose analogs is not reduced. This is in contrast to 'normal' 3T3 cells which exhibit decreased transport rates for these same compounds. This suggests that hexose sugar and uridine uptake changes occurring during G1-arrest of 3T3 cells may be G0-specific events, or that the G1-arrest states in normal and transformed cells have different characteristics.

The H6-15 subline of SV-3T3 cells is a temperature-sensitive derivative which demonstrates normal growth behavior at 39 degrees C, but transformed behavior at 32 degrees C. 3-0-methylglucose transport is 2-4-fold higher when the cells are grown at 32 degrees C. However, at both the restrictive and permissive temperatures there is a marked cell density-dependent reduction in uptake. This seems inconsistent with hexose sugar transport activity being a determinant of growth potential of transformed cells. In addition, preliminary experiments indicate a 3-4-fold difference in serum component-depletion activity when cells are grown at 32 degrees C. This is consistent with the hypothesis that transformed cells have reduced serum requirements for growth.

511. DIETARY DISTRIBUTION AND METABOLIC FATE OF TRANS FATTY ACIDS

Keeney, M., Sampugna, J., Munn, R. J., University of Maryland, College Park Campus, Agricultural Experiment Station, Dept. of Dairy Science, *College Park, Maryland*, 20740, United States of America

OBJECTIVE: Determine fatty acid composition, particularly Trans-and Cis- acids in the American diet; alterations in membrane structure resulting from incorporating trans fatty acids; the transport of trans fatty acids into brain tissue; whether trans fatty acids are transported across placental membranes, and conduct an epidemiological study comparing intake of trans fatty acids by 3 groups with different risk of cancer.

APPROACH: By standard methods determine fatty acid content of dietary fats and oils. Evaluate lipid from many tissues of organs for mice fed control diets and diets high in trans fatty acids. Similar studies to 2 on brain tissue. Evaluate lipids in the fetuses from cattle and mice fed diets high in trans fatty acids. Evaluate kind of fat eaten by a Jewish (high risk) and Seventhe Day Adventist (SDA) (low risk), and a non-Jewish-non-SDA population (control). Compare trans fatty acid levels in these diets.

PROGRESS: Using glass capillary gas-liquid chromatography, more than 200 food items have been analyzed for fatty acid composition with emphasis on the trans-octadecenoate and trans-octadecadienoate content. Based upon these analyses, menus developed to comply with the McGovern Committee Dietary Goals, Prudent Diet, or Alternative Diet were calculated to contain increased levels of trans-fatty acids compared to current estimated intake levels. A paper summarizing this work will be presented at the ISF/AOCS World Congress Meetings in New York City (April 27-May 1, 1980). We have also initiated a number of dietary studies using mice as experimental animals. Thus far, we have investigated the transfer of trans-fatty acids from mother to fetus and the influence of a margarine-containing diet on aryl hydrocarbon hydroxylase activity. The results of these studies, which will be presented in detail at the ISF/AOCS Meetings, indicate that: (1) Trans-fatty acids may be present in tissues of mice, even though they are on diets with no

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apparent trans-fatty acid components. (2) Trans-fatty acids are transferred from mother to fetus. (3) Compared to control or chow diets, those containing margarine fat result in elevated aryl hydrocarbon hydroxylase activity in the microsomal fraction of mouse liver.

512. BIOPHYSICAL STUDIES OF TISSUE FUNCTION AND CELLULAR STRUCTURE ASSOCIATED WITH MALIGNANCY

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OBJECTIVE: To investigate, using the electron spin resonance (ESR) technique, the role of endogenous paramagnetic species in: (a) cell transformation and (b) iron transport.

APPROACH: (a) Cell transformation: Cell systems which transform with a high incidence over a limited period of time, e.g., green monkey kidney cells, BALB/c mouse fibroblasts, are examined for changes in concentration of paramagnetic species. (b) Iron transport: Intermediates involved in the transfer of iron are examined using cell lines such as Friend erythroblasts or Chang cells in which uptake of iron can be controlled.

PROGRESS: Changes in the concentration of paramagnetic species, both free radicals and metal ions, have been observed in cells in vitro during the early stages of malignant transformation. The significance of these changes and their role in carcinogenesis are being investigated further. Changes have also been observed in the nature and concentration of certain iron containing intermediates in Friend cells following induction of hemoglobinization. The nature of these intermediates is being investigated in order to clarify the pathway by which iron is transported from serum transferrin to cellular hemoglobin. Further studies on the intracellular labile iron pool are being carried out using iron loaded Chang cells.

513. BIOCHEMICAL STUDIES ON THE GAMMA-GLUTAMYL CYCLE AND GLUTATHIONE METABOLISM IN TUMORS

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We plan to continue research on the enzymes of the gamma-glutamyl cycle, and related enzymes that are involved in the biosynthesis and utilization of glutathione. There is evidence that the gamma-glutamyl cycle is involved in transport and membrane phenomena. Our work will focus on gamma-glutamyl transpeptidase, the translocation of glutathione across cell membranes, transport of gamma-glutamyl amino acids, and gamma-glutamyl cyclotransferase. Work in this laboratory showed that gamma-glutamyl transpeptidase is localized on the cell surface of lymphoid cells; further studies will be concerned with the biological function of this enzyme and its modulation in relation to blastogenesis and neoplasia. Glutathione and gamma-glutamyl transpeptidase seem to play a role in carcinogenesis in the liver; high levels of glutathione have been reported in skin and colon carcinomas. Studies will also be carried out on other aspects of glutathione synthesis and utilization in tumors, including the conversion of glutathione to glutathione disulfide.

514. TRANSPORT STUDIES OF NORMAL VS MALIGNANT CELLS

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This is part of a broader project. A summary of this subproject is not available.

515. TRANSPORT - POLARIZATION IN COLUMNAR EPITHELIUM (RATS)

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This is part of a broader project. A summary of this subproject is not available.

C. ION TRANSPORT AND ITS ENERGY DEPENDENCE IN NORMAL AND NEOPLASTIC CELLS

516. AMPHOTERICIN B INCREASES NA ION ENTRY AND NA ION-K ION PUMP ACTIVITY IN QUIESCENT 3T3 CELLS

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Monensin, a monocarboxylic acid which forms a cage-like structure with monovalent cations, markedly increases the activity of the Na ion-K ion pump (4-6 fold) in Swiss 3T3 cells. The acceleration of 86Rb ion entry by monensin has an absolute requirement for external Na ion and does not occur if ouabain is present to block the pump (Smith and Rozengurt, Proceedings of the National Academy of Science, USA, 1979, 75, 5560). It was possible that the effect of monensin on the Na ion-K ion pump resulted from some effect of the ionophore other than the increased intracellular Na ion concentration. This seemed unlikely since ouabain-sensitive 86Rb uptake was also stimulated by gramicidin, which has equal affinities for Na ion and K ion but not by the K-ionophore valinomycin or the divalent cation ionophore A23187. To support further the hypothesis that the effect of monensin was caused by its effects on Na entry into the cell, we studied the effect on 86Rb uptake and intracellular ion content of the polyene antibiotic amphotericin B which causes a generalized increase in membrane permeability by interacting with sterols in the cell membrane. Amphotericin B stimulated total 86Rb uptake as well as ouabain-inhibitable 86Rb uptake. The effect of amphotericin B on 86Rb uptake was steeply dose-dependent with maximal stimulaton occuring at concentrations of about 0.5 micrograms/ml and inhibition of 86Rb uptake occuring at higher concentrations of amphotericin B. We verified that the antibiotic causes an increase in cellular Na ion and Na/K in Swiss 3T3 cells. These findings provide further support for the hypothesis that intracellular Na ion plays a role in the regulation of the Na ion-K ion pump activity.

517. SERUM STIMULATES NA ION INFLUX AND PUMP ACTIVITY IN MURINE, HAMSTER AND HUMAN CELLS

Mendoza, S., Wigglesworth, N. M., Rozengurt, E., Imperial Cancer Research Fund, Membrane Physiology & Growth Regulation Laboratory, *Lincolns Inn Fields, London WC2A 3PX, England, United Kingdom*

The cell membrane Na ion-K ion pump of Swiss 3T3 cells is sensitive to fluctuations in internal Na ion. The pump activity in intact cells, as measured by the rate of 86Rb ion influx, increases several-fold when certain ionophores are used to raise internal Na ion (Smith and Rozengurt, Proceedings of the National Academy of Science, USA, 1979, 75, 5560). Since the regulation of the Na ion-K ion pump by intracellular Na ion concentration may play a significant role in the modulation of the pump by growth-promoting factors, we decided to study the generality of the relationship between Na ion concentration and the activity of the pump. We found that elevation of intracellular Na ion concentration elicited by the monocarboxylic acid Na ion ionophore monensin leads to activation of the Na ion-K ion pump in cells of murine (Swiss 3T3, Balb 3T3, 3T6, tertiary cultures of whole mouse embryos), hamster (Nil, BHK) and human (GM-75, En) origin. Furthermore, addition of serum stimulates ouabain-sensitive 86Rb ion uptake (and DNA synthesis) in quiescent cultures of each of these cell types. The magnitude of the stimulation of 86Rb ion uptake by monensin in a particular cell type correlates significantly with the magnitude of the stimultion of 86Rb ion uptake by serum in that cell type. This correlation supports the contention that serum and monensin stimulate the Na ion-K ion pump in the same manner, namely by increasing intracellular Na ion concentration. These findings are all consistent with the proposal that Na ion entry plays a critical role in the regulation of the Na ion-K ion pump and suggest that this mechanism of control may have wide significance.

518. RESPIRATION-COUPLED TRANSPORT PROCESSES IN TUMOR CELLS

Lehninger, A. L., Reynafarje, B., Granger, D., Vercesi, A., Fiskum, G., Lozano Chavez, C., Bruhn, H., Johns Hopkins University, School of Medicine, Dept. of Physiological Chemistry, *725 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

Our specific objectives for the coming year are directed to the respiration and bioenergetics of cancer cells, particularly the stoichiometry and efficiency of energy coupled H ion movements, ATP synthesis, and Ca2 ion transport: (1) We propose to continue our analysis of the stoichiometry of H ion ejection by respiring tumor mitochondria, particularly the H ion/O ratio of the cytochrome oxidase reaction, using a new type of oxygen electrode developed in this laboratory and fast recording techniques, using a recording cathode ray oscillograph. (2) We shall also determine the H ion/ATP stoichiometric ratio during the breakdown and synthesis of ATP by the F0F1ATPase activity of intact tumor mitochondria. (3) We propose to determine the stoichiometric relationships between the movement of ATP, ADP, and phosphate across the mitochondrial membrane during both oxidative phosphorylation and energy dependent reversal of electron transport. (4) In selectively permeabilized intact tumor cells we propose to determine the H ion/O ratio of mitochondiral electron transport in situ, the P/O ratio of oxidative phosphorylation in situ, and the stoichiometry of Ca2 ion transport. (5) We propose to test further our hypothesis, based on evidence derived from our earlier work, that the rate of ATP formation in intact tumor cells is depressed because of an abnormal affect of intramitochondrial Ca2 ion on ATP translocation or synthesis.

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519. SODIUM TRANSPORT IN GLUCOSE ABD HORMONE REGULATION

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With a membrane potential sensitive probe in the isolated adipocyte, we have noted membrane depolarization and hyperpolarization with catecholamines and insulin, respectively. These responses were correlated with the lipolytic and antilipolytic activity of these hormones. Preliminary studies of the ionic determinants of hormonal responses implicate sodium in the membrane potential and glucose transport response of catecholamines. As a working hypothesis we suggest that Na ion redistribution leads to a change in structure and function of several relevant membrane components. We shall study the effects of Na ion and membrane potential on basal and catecholamine influenced glucose transport, membrane potential, and catecholamine and insulin binding. Glucose transport will be evaluated with the non-metabolizable glucose analogues, 2-deoxy glucose and 3-0-methyl glucose. Membrane potential will be determined with the membrane potential probe triphenylmethylphosphonium. The numbers and affinities of the catecholamine and insulin receptors will be studied with radioactive dihydroalprenolol and hydroxybenzylpindolol and insulin respectively. The glucose transporter will be labeled with (14C)-cytochalasin B. Determinations will be made with the intact isolated adipocyte as well as the plasma membrane derived therefrom. These studies should contribute to an understanding of the role of ion redistribution and membrane potential to hormonal receptor activity, receptor-effector coupling and effector activity.

520. SODIUM ION FLUXES AND MITOGENIC SIGNALING

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It is not known how any mitogenic peptide or tumor promoter activates a quiescent cell. In 1978, we reported evidence that certain peptide growth factors act by rapidly increasing Na permeability of the quiescent cell. Transformed cells in culture apparently contain more Na and/or are more permeable to Na than non-transformed ones. The greater permeability to Na probably causes the increase in Na-K pump activity which occurs soon after mitogenic activation and, at least in part, the greater pump activity in some transformed cell lines. Recently we reported that the enhancement of Na-dependent amino acid transport, which is also highly correlated with the activation of the quiescent cell in vitro, may in turn be due to the hyperpolarizing effect of pump stimulation since the pump is electrogenic. We plan to further explore the hypothesis that an increase in cell permeability to Na plays an important role in transformation, tumor promotion, and mitogenic signal transduction. Secondary changes in free Ca or protons may be responsible for altering energy metabolism and macromolecular syntheses. We call this hypothesis stimulus-division coupling since the proposed alterations in cation transport are analogous to the ones involved in muscle contraction and secretion.

The activity of the Na specific and non-specific cation transporters will be assayed as a function of cell density and growth state in non-transformed and transformed fibroblasts, by following both Li and 22Na ion entry and exit. Effects of internal and external Ca and MG ions, purified plasma membranes, individual peptide mitogens and tumor promoters, e.g., vasopressin and phorbol esters, will be examined. Amiloride-derivatives will be tried as irreversible inhibitors and photo-affinity labels of the amiloride-sensitive Na transporter. Growth of fibroblastic and two mammary carcinoma cell lines, in completely defined media, will be used to look for specific antagonists of the mitogenic response to vasopressin, phorbol esters and other tumor promoters. Na transport inhibitors, retinoids, selenium, Li and Rb ions will be tried. The mechanism and regulation of Na and Ca transport will be studied in the MCF-7 and ZR-75-1 lines of mammary carcinoma cells and in purified membrane vesicles from fibroblastic cells.

521. PHOSPHATE TRANSPORT AND METABOLISM IN CARCINOMA CELLS

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Previous work in our laboratory has led to the conclusion that in the Ehrlich-Lettre ascites tumor cell both chloride and sulfate are transported by a common mechanism possessing two reactive membrane sites. This conclusion is based on: 1) the effect of sulfate on chloride transport and chloride on sulfate transport; and 2) the effect of H2DIDS (4,4'-diisothiocyano-1,2 diphenyl ethane, 2,2'-disulfonate) on the transport of both anions. Reversibly bound H2DIDS inhibits transport of sulfate to a greater extent than chloride. However, covalently bound H2DIDS inhibits transmembrane movement of sulfate but is without effect on chloride. This suggests that H2DIDS interacts irreversibly with that component of the anion transporter concerned with the regulation of sulfate permeability. We have recently synthesized tritium labeled-H2DIDS and have determined the relationship between 3H2DIDS binding and inhibition of sulfate transport. Two membrane proteins, 93K and 99K MW, have been implicated in the transport process. In the coming year we plan to establish the Ehrlich cell in tissue culture and measure the turnover rate of the membrane proteins.

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522. ALTERATION OF MONOVALENT ION TRANSPORT IN TRANSFORMED CELLS 3T3

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The possibility that changes in monovalent ion trasport are involved in the regulation of cell growth prompted the study of ion fluxes by transformed cells. Several workers have previously reported that 86Rb uptake is increased in transformed cells, but measurement of Na ion-K ion ATPase activity from homogenates of transformed cells has given variable results. We have confirmed the increase in 86Rb uptake in Swiss 3T3 cells transformed by polyoma or SV40 virus. One possible explanation for the failure to demonstrate a consistent change in Na-K ATPase activity in spite of the increased 86Rb uptake is that the activity of the pump is not intrinsically increased but rather that the pump is more active because of an increased availability of Na due to increased Na permeability. This possibility was studied by following changes in intracellular electrolytes in normal and transformed cells after the addition of ouabain. Two minutes after the addition of 1mM ouabain, cellular Na ion and K ion concentrations in growing Swiss 3T3 cells are essentially unchanged. In contrrast, cellular Na ion concentration increases significantly during a 2-minute exposure to ouabain in Swiss 3T3 cells transformed with either SV40 or polyoma virus. Finally the rate of 22Na uptake is 2-3 fold higher in SV40 or polyoma virus-transformed Swiss 3T3 cells than in growing 3T3 cells. These observations taken together suggest that Na permeability is markedly increased in transformed cells and that this results in increased activity of the Na ion-K ion pump.

523. MEMBRANE PERMEABILITY OF THE ASCITES TUMOR CELLS

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The long-range objective of this research is to elucidate the relationship between cell membrane structure and the cell membrane functions of solute transport and permeability, particularly as this relationship is expressed in a malignant cell, the Ehrlich mouse ascites tumor cell. The goal for this grant period is to define the action of probes which inhibit chloride transfer, prior to initiating biochemical investigations to identify membrane constituent(s) which mediate chloride movement. By focusing on chloride transfer, which is being studied extensively with physiologic and biochemical methods in human erythrocytes, we can learn about membrane differences between that cell and a rapidly proliferating mammalian eukaryotic cell. Studies will assess whether inhibitory probes interact specifically with a chloride transport system and will determine which probes will be most suitable for future biochemical labeling experiments. Experiments to determine the mechanism of action of furosemide will continue; this diuretic drug substantially inhibits one for one chloride exchange and its effect is partly irreversible. A number of other small chemical probes will be assessed for their effectiveness under different conditions, their specificity, reversibility, and mechanism of action. In order to characterize clearly the transfer process and the mechanism of probe action, procedures will be developed to investigate chloride flux in the absence of competing anions. This will allow determination of whether there is saturation behavior in the relationship between chloride flux and chloride concentration and the way in which probes affect this relationship; of whether or not there is competition of chloride and a probe for a common membrane site; and of the magnitude of chloride conductance (net flux) versus self-exchange and of the effects of a probe on each. The action of probes on transfer of ions like potassium and sulfate will be determined to help evaluate specificity.

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524. MEMBRANE TRANSPORT ADAPTATIONS BY HUMAN LYMPHOCYTES

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First, we will further define the alterations in membrane permeability that occur after lectin treatment of lymphocytes. We will examine the role of a subtle increase in internal sodium (Na) (approximately 5mM) as the cause of an increased active transport rate of Na and potassium (K) in lymphocytes treated with lectins. We will 1) measure the Na content of lectin-treated lymphocytes, 2) determine the Na and K flux rates of untreated lymphocytes at an internal Na found in lectin-treated cells and 3) measure the kinetics of the transport-adenosine-triphosphatase of lymphocyte membrane vesicles for its cofactor Na. Second, we propose to define calcium (Ca) metabolism in lectin-treated and calcium-ionophore treated lymphocytes. These studies will measure the permeability, exchange and transport, of Ca by the membrane of cells and vesicles and isolated mitochondria. We will study the perturbation of Ca content, distribution and movement produced by lectin and ionophore treatment and correlate these with mitogenicity of these chemicals. Third, we plan to define the uptake of natural, neutral and amino acids by lymphocytes and characterize the alterations in membrane transport of natural amino acids induced by lectins or ionophores. In these studies, we propose to define the A and L-systems of amino acid transport and their alterations after perturbation by lectins or ionophores. Alterations in transport will be compared with the changes that occur when lymphocytes are exposed to amino acid deficient medium. We will determine if adaptation and lectin-induced enhanced amino acid uptake are similar. A vs. ASC vs. L-system amino acid uptake, kinetic parameters of uptake and sensitivity to metabolic inhibitors and glycocorticoids will be studied. We will define the differences in these membrane properties of human blood B and T lymphocytes.

525. REGULATION OF THE CELL CYCLE AND DIFFERENTIATION OF NEUROBLASTOMA CELLS IN VITRO - ROLE OF ION TRANSPORT

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OBJECTIVE: To reveal the molecular mechanisms underlying the regulation of growth and differentiation of neuroblastoma cells, in particular the possible role of the plasma membrane ion transport properties.

APPROACH: The intrinsic modulations in cation transport properties, including the plasma membrane-bound (Na plus-K plus)-ATPase, are studied by tracer-flux methods and biochemical techniques. The possible role of these membrane properties in the regulation of the cell cycle and differentiation is established directly by modification of the cation transport properties, and indirectly by experimental modification of the plasma membrane composition.

526. CONTROL MECHANISMS OF ION AND ELECTRON TRANSFER AT THE MEMBRANE INTERFACE

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This is part of a broader project. A summary of this subproject is not available.

527. REGULATION OF CELL GROWTH BY MEMBRANE ION FLUX

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Increased flux of Na and K ions across the cell membrane with a resultant increase in intracellular K ion concentration has been proposed to play a critical role in the regulation of cell proliferation (Rozengurt and Heppel, 1975). The present study will demonstrate if this increased ion flux is a necessary prerequisite for replication of Balb/c-3T3 cells. In addition, regulation of ion flux by the cell growth stimulating factors from serum, platelet-derived growth factor (PDGF), and somatomedin, as well as by the tumor promotor tetradecanyol phorbol acetate (TPA) and by the oncogenic virus SV40 will be explored. The effects of these agents on ion flux will be studied both in whole cells and in membrane preparations and membrane vesicles from cells arrested at several defined 'points' in the G0/G1 phase of the cell cycle. Systematic analysis of direct effects of these agents on ion flux in membrane vesicles from the growth-arrested cells will define the roles of Na-K ions ATPase and other ion transport mechanisms in the regulation of cell growth.

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528. MECHANISM OF CATION TRANSPORT REGULATION

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The objective of this research is to determine the mechanism by which peptide hormones (such as insulin) stimulate active or passive cation transport across the plasma membrane and secondly to determine whether regulation of cation transport plays an essential role in altering cellular metabolism or differentiation. Two different approaches will be taken toward solving these problems. In the first case, we will obtain a well defined subcellular system which shows insulin regulation of an enzymatic reaction. In particular we will: 1) Attempt to verify insulin inhibition of Ca2 ion-ATPase activity of hepatocyte plasma membranes, 2) Attempt to verify stimulation of mitochondrial pyruvate dehydrogenase by addition of insulin plus fat cell plasma membranes, 3) Determine the minimum requirements of these two systems and use these sub-cellular systems to purify and identify any essential proteins and/or small molecules, 4) Reconstitute plasma membrane vesicles capable of insulin regulated Ca2 ion transport.

The second part of this research will be concerned with determining whether an alteration in cation transport is an early and essential event for induction of murine erythroleukemia cells to differentiate into red cells. We intend to test the hypothesis that inducing agents (such as DMSO) act by causing a transient dissipation of the plasma membrane Na ion gradient and/or membrane potential to allow increases in cytoplasmic Ca2 ion concentrations which are essential for later events in differentiation.

529. EXTRINSIC FLUORESCENCE AND MEMBRANE POTENTIALS

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The general goal of this project is to demonstrate that fluorometric methods may be used to monitor membrane potentials in a eucaryote cell, the Ehrlich ascites tumor cell.

We are studying the membrane potential in conjunction with measurements of passive ion fluxes, co-transport and active ion transport, in order to develop a quantitative expression for the potential difference across the membrane of this cell. These measurements, and similar studies with membrane bound cytoplasts of the cells, are being used to test the hypothesis that the energy for Na-dependent amino acid transport is derived solely from the electrochemical gradient of Na ion (the Na ion gradient hypothesis). We are developing the cytoplast preparation to avoid the complications of assessing the Na ion gradient if the nucleus sequesters Na ion.

The influence of the membrane potential is also being considered in other aspects of Na ion-dependent amino acid transport, including stoichiometry and the possible involvement of K ion.

530. ATP-LINKED TRANSPORT IN MICROORGANISMS

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There is now strong evidence that in bacteria the linkage between major metabolic pathways and transport systems for metabolites is indirect, effected by a circulation of protons across the cytoplasmic membrane (chemiosmotic theory). At the same time we have come to recognize a second major class of transport systems that are energized by a phosphoryl donor, possibly ATP. Indeed, some important systems, including those for monovalent cations of K and N and monovalent anion of Pi, require both ATP and the proton circulation. It is entirely possible that ATP-linked transport systems will prove to be widespread in eukaryotic organisms as well. The molecular basis of ATP-linked transport is virtually unknown. I plan to study the movements of monovalent cations of K and Na, divalent cation of Ca, monovalent anion of Pi and glutamate in intact cells and membrane vesicles of Streptococcus faecalis, an organism that offers technical as well as conceptual advantages. The objectives are to determine the chemical identity of the phosphoryl donor in each case, the role of the 'ATP' in the transport process, and to unravel the interactions between 'ATP' and the proton circulation. Subsequent lines of inquiry include the search for ATP-linked transport systems in eukaryotic microorganisms.

531. ROLE OF SERUM AND CATIONS IN MALIGNANT CELL GROWTH

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Several observations indicate that the different effects of serum and serum components on normal versus transformed cell growth are related to divalent cation metabolism. The growth of normal cells in culture can be modified by the availability of both serum and divalent cations. There is evidence that divalent cations and serum act synergistically in the control of cell growth and that this control is lost upon transformation to the malignant state. Serum or calcium deprivation inhibits proliferation of a number of normal cells in contrast to the absence of such an effect on transformed counterparts. However, elevation of medium Ca levels in serum deprived normal cells can antagonize the growth inhibitory effect of low serum. The loss of density dependent growth inhibition in 3T3 cells is characterized by a simultaneous reduction in the extracellular Ca and serum requirement. Magnesium and serum act synergistically in inducing chicken fibroblasts to traverse G1 phase and initiate DNA synthesis. Serum platelet factors are required to commit several normal cell types - but not transformed cells - to DNA synthesis. Ca can substitute in part for these factors. We propose to establish the extracellular Ca, Mg and serum requirements for growth of human diploid fibroblasts as compared to an SV40 transformed counterpart with specific reference to their ability to traverse G1 phase of the cell cycle, the phase in which growth control is exerted in the normal cell. We have achieved synchronization of both normal and transformed W138 cells using centrifugal elutriation. This will allow direct comparisons to be made between these cells using an identical synchronization procedure. We propose to evaluate what effects the manipulation of serum, Ca and Mg have on divalent cation transport and content during G1 phase progression in the normal cell and how these parameters are related to loss of growth control in the transformed cell. We propose to determine any specific changes in divalent cation management associated with the ability of platelet factor to induce competence for DNA synthesis in normal cells and to establish how such changes might relate to the capability of transformed cells to remain continually competent in the absence of platelet factor.

532. ENERGY METABOLISM IN NORMAL AND TUMOR CELLS

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It is planned to study the mechanism and control of energy transducing processes in normal and tumor cells. Three ion pumps and several channels will be investigated. The proton translocating pump of mitochondria, the Ca 2 ion pump of sarcoplasmic reticulum, and the Na ion K ion pump of the plasma membrane are the ATP driven pumps that will be analyzed. Specific emphasis will be placed on the phosphorylation process of the beta subunit of the Na ion K ion ATPase which we have shown renders the pump inefficient in tumor cells. The studies on the proton pump of mitochondria will be continued with emphasis on the purification and characterization of the 28,000 dalton subunit of the hydrophobic sector. Pathways of Ca 2 ion fluxes will be analyzed at the level of the plasma

membrane, the sarcoplasmic reticulum and the mitochondria. Further attempts will be made to resolve the acetylcholine receptor and to analyze the individual subunits. We shall try to define the lipid requirements of this channel with emphasis on the neutral lipid which we have shown to be required.

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533. THE EFFECT OF COCARCINOGENS ON CELLULAR MEMBRANES

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Early events of tumor promoting phorbol esters are changes in properties of cell membranes which are considered of crucial importance in the regulation of cell growth and differentiation. We have observed that the potent tumor promoter, 12-O-tetradecanoyl-phorbol-13-acetate (TPA) stimulates the plasma membrane (Na ion plus K ion)-ATPase, and it is of interest to learn whether this effect is mediated by intracellular Ca2 ion release (possibly from membrane-bound Ca2 ion), or changes in membrane potential, and whether this stimulatory effect is shared by retinoids, beta-receptor agonists, or intermediates of arachidonic acid metabolism. Therefore TPA-stimulated 86Rb ion uptake will be examined in the presence of agents which interfere with each of these processes, e.g. chloropromazine, beta-adrenergic antagonists, and inhibitors of lipoxgenase activity. Further, it will be of interest to see whether Ca2 ion efflux is promoted by TPA under conditions of preloading mouse fibroblasts with 45Ca2 ion, and to learn with the use of radioautography, changes in Ca2 ion distribution.

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534. ENERGY-COUPLING PROCESSES IN MITOCHONDRIA

Lehninger, A. L., Reynafarje, B., Tew, W. P., Beavis, A., Decker, G., Alexandre, A., Moreadith, R., Johns Hopkins University, School of Medicine, Dept. of Physiological Chemistry, *725 N. Wolfe St., Baltimore, Maryland*, 21205, United States of America

We propose to continue our efforts to identify the specific electron carriers of the respiratory chain that are responsible for translocating electric charges and H ion across the mitochondrial membrane, thus generating an energy-rich electrochemical gradient which is used in the synthesis of ATP. In particular, we shall concentrate on energy-conserving site 3, that promoted by cytochrome oxidase, since our investigations indicate that this is the key enzyme in the respiratory chain responsible for regulating the overall rate of H ion extrusion and oxidative phosphorylation. We also propose to examine the transport mechanisms in the inner membrane that bring about H ion translocation, in order to determine whether the primary gradient employed in oxidative phosphorylation is formed between two bulk phases or whether it is generated within or on the inner membrane itself.

We also propose to examine the membrane transport menchanisms for ATP, ADP and phosphate that participate during oxidative phosphorylation and during ATP-dependent reverse electron flow, which is an important regulatory process in the coordination of glycolysis and respiration. We also propose to investigate the regulation of Ca2 ion influx and efflux by respiring mitochondria and how Ca2 ion homeostasis serves as a signaling agent in the bio-energetic relationships between the cell cytosol and the mitochondria.

BIBLIOGRAPHIC REFERENCES: Lehninger, A. L., Reynafarje, B., Alexandre, A., and Villalobo, A., Respiration-coupled H ion ejection by mitochondria, Annals of the New York Academy of Sciences, Vol. 341, 585 (1980). Fiskum, G. and Lehninger, A. L., The mechanisms and regulation of mitochondrial Ca2 ion transport. Symposium on Ca2 ion Pumps. Fed. Proc. 39, 2432 (1980).

535. METABOLIC REGULATION IN NEOPLASMS BY CATION TRANSPORT

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The broad objective is to understand the relationship between cellular metabolism and a) membrane ionic permeabilities, b) transmembrane ionic gradients and c) subcellular compartmentation of various ions. Mitochondrial cation transport will be stressed, particularly Ca2 ion and the distribution of metabolites between mitochondrial and cytoplasmic compartments. The possible interplay between mitochondrial Ca2 ion and Na ion transport will be studied to determine whether the translocation of Na ion may regulate the levels of Ca2 ion within the cytosol. The experimental approach will involve comparative studies of isolated cells and mitochondria to detail regulatory influences. Intact cell studies will involve alteration of intracellular ion levels together with specific alterations in the permeability of membranes with monovalent and divalent cation specific 'ionophores'. The influence of certain hormones upon membrane permeability and metabolism will form part of the above studies. The studies outlined will be applied to isolated liver cells in comparison to a selected variety of tumor cells to determine the extent to which the indicated ions and their translocation between compartments affect the oxidative and glycolytic metabolism of tumor cells, as well as relevant hormonal influences.

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D. OTHER STUDIES OF THE CELL MEMBRANE AND TRANSPORT MECHANISMS IN NORMAL AND TUMOR CELLS

536. INVESTIGATION OF THE TRANSLOCATION OF GLUTATHIONE FROM LYMPHOID CELLS

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The main objective of this proposal is to analyze the process whereby intracellular glutathione is translocated across the plasma membranes for extracellular utilization. Recently, it was established that the observed export was not a consequence of membrane damage. No other details of the translocation process have been investigated under physiological conditions. Since participation by glutathione in the transport of amino acids via the gamma-glutamyl cycle is probably not the only function glutathione performs, a correlation between the transport (rate and regulation) of glutathione and the fibronectin-dependent ability of cells to adhere to each other and an artificial surface is suggested. Two approaches are proposed. First, a kinetic analysis of the translocation phenomena will provide an understanding of the translocation mechanism (i.e., simple diffusion or mediated transport). An investigation of the factors which may regulate the rate of translocation, such as energy requirements and phase of the cell cycle, will indicate whether the export is active transport and whether the rate of export may be correlated to cell division and metabolism. Secondly, observations of and rate of support of glutathione will indicate whether glutathione functions to regulate cell adhesion phenomena.

537. PEPTIDE TRANSPORT IN EHRLICH ASCITES TUMOR CELLS

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OBJECTIVES: The hypothesis forming the foundation of this research prgram is that cancer cells are cancerous because they no longer respond to chemical (hormonal) regulatory signals in their environment. Peptide hormones constitute one major class of regulatory molecules and if a tumor cell has suddenly acquired the ability to transport the smaller peptide hormones it may no longer respond to that peptide as a regula-

tory molecule. Hence our objective is to characterize the response of tumor cells to small peptides in their environment: Do cancer cells transport small peptides?

APPROACH: The approach is quite simple. We incubate tumor cells in the presence of di, tri, up to penta peptides and measure the rate of accumulation of the peptide by the tumor cells. (Wolfinbarger, Jr., L. and G.A. Marzluf. 1975. Arch. Biochem. Biophys. 171:637.)

538. TRANSPORT OF PROTEINS ACROSS MITOCHONDRIAL MEMBRANES

Ching, E. P., Rockefeller University, Lab. of Cell Biology, *66th & York Ave., New York, New York*, 10021, United States of America

I propose to work on the transport of cytoplasmic subunits of mitochondrial enzymes through the double mitochondrial membranes in yeast. The mitochondria are a system where a transport mechanism other than the signal hypothesis is reasonably suggested. The investigation will determine the presence or absence of a hydrophobic amino terminal sequence on the primary translation product of specific cytoplasmically synthesized but intramitochondrially localized enzymes. This will be accomplished by preparation of immunoprecipitated primary translation products synthesized by an in vitro protein synthesizing system and comparison with the mature forms of the proteins. Thereafter, the kinetics of transport and the specific components of the transport mechanism or its specificity will be studied.

539. POST-TRANSLATIONAL TRANSPORT OF PROTEINS ACROSS BIOLOGICAL MEMBRANES

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The goal of the proposed resarch is to elucidate the molecular mechanism(s) by which a specific protein, which is synthesized in the cytoplasm, is targeted to and transported by the particular organelle within which it will reside. To achieve this objective, I plan to study a well-characterized mitochondrial protein, the polyprotein precursor (Pr IV-VII) to subunits IV-VII of yeast (Saccharomyces cerevisiae) cytochrome c oxidase, from its site of synthesis in the cytosol to its permanent location in the inner mitochondrial membrane. By using anti-polyprotein antibody, in vitro translation and transport systems, and 'mitochondrial protein transport' defective yeast mutants, I hope to: 1) identify any pre-polyprotein precursor(s), 2) define the intracellular transport pathways followed by the polyprotein, and 3) examine the protein components of the transport system(s) involved in importing the polyprotein into and across mitochondrial membranes.

540. STUDIES ON THE PASSAGE OF PROTEINS THROUGH MEMBRANES

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The signal sequence of secreted proteins is essential for the secretion process. However, evidence exists that other features of the sequence of such proteins are necessary for them to pass through biological membranes. Studies on hybrid proteins in which a signal sequence is attached to the normally cytoplasmic protein beta-glactosidase indicate that this protein may be incapable of being secreted through a lipid bilayer. I intend to isolate mutant strains in which the gene for beta-glactosidase is altered so as to allow the secretion of the protein when a signal sequence is attached to it. In this way I will be able to genetically define those portions of the protein which present a barrier to secretion.

541. NEW LABELS FOR STUDYING MEMBRANE PROTEINS

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Study of membrane proteins is an important segment of the long range goal of understanding at the molecular level how cells function. The central effort of this envisaged five year program of research is the design, chemical synthesis, and characterization of an array of broadly applicable, new and unique labels for biological membrane studies, with particular emphasis on membrane proteins. In a continuing collaboration with Drs. O.H. Griffith and P.C. Jost at the University of Oregon, these new labels will be used in on-going biochemical and biophysical studies of the properties of beef heart mitochondrial cytochrome oxidase, shark rectal gland Na ion/K ion-ATPase, sarcoplasmic reticulum Ca2-dependent ATP-ase, high density lipoprotein (HDL), as well as other lipid requiring proteins. Examples of envisaged new labels include several unique turn-on-able amino acid specific membrane protein labels, development of novel free radical based membrane protein photolabels, development of a series of diamagnetic isopolar, isosteric structural analogues of doxyl and proxyl nitroxides to be used to explore questions of steric perturbation inherent with the spin labeling method and to determine equilibrium binding constants at specific protein sites, if any, and the development of new heterobifunctional nitroxides potentially useful for saturation transfer ESR spin label studies.

542. FLUORESCENT PROBE STUDY OF MEMBRANES

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To help elucidate the structure and function of biological membranes we will study the physical properties of the lipid and protein components of membranes using fluorescent probes. Techniques are being developed to obtain information on the tertiary structure and function of the hydrophobic region of intramembranous proteins. Model membranes will be used to establish the correlation between fluorescent probe response and membrane structure. This study will be applied to the investigation of a human erythrocyte membrane preparation whose protein composition consists only of band 3. By means of energy transfer between the tryptophan located in the hydrophobic region of this protein and derivatives of anthroyl fatty acids dispersed in the lipid phase, the distribution of the protein's tryptophan residues will be mapped out. We have used this preparation and our preliminary results indicate that the tryptophan residues are probably located near the center of the bilayer. Once the distribution is established, we will attempt to determine the conformation changes associated with anion transport by monitoring alterations in the tryptophan distribution.

543. COMPOSITION AND PROCESSING OF THE PINOCYTIC VESICLE MEMBRANE

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The goal of this project is to compare the protein composition of the pinocytic vesicle membrane with that of the plasma membrane and to monitor the processing of the pinocytic vesicle with time. Briefly, we will use a cleavable, photo-activated dextran derivitive to label the membranes of cells of the J774.2 macrophage line which have been pre-incubated in 35S-methionine. The dextran-labeled proteins will be separated, subjected to electrophoresis, and detected by autoradiography. This technique should allow separate labeling of the pinocytic vesicle and plasma membrane proteins, as well as providing sufficient time resolution to permit observation of temporal changes in composition.

544. LIPID TRANSPORT AND METABOLISM IN CANCER-HOST SYSTEMS

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We wish to extend our earlier studies of the rapid fatty acid (FA) esterification-hydrolysis cycle that we have described in Ehrlich ascites carcinoma and to explore other aspects of tumor-host lipid transport and metabolism that have not been adequately studied because of their highly complex and difficult nature. First, we shall try to establish whether the FA esterification-hydrolysis cycle is more rapid in malignant than in non-malignant tumors or normal tissues. By studying this cycle both in vivo and in vitro we may be able to suggest new strategies relevant to diagnosis and treatment of cancer. We also wish to

test current hypotheses regarding the effect of cancer growth on host lipid metabolism. We will continue to explore the possible existence of unique or altered mechanisms of lipid transport to the carcinoma. In particular, we shall extend our earlier hypothesis that cancer cells can induce rapid lipolysis in adjacent adipose tissue and the direct delivery of free fatty acids to the carcinoma. We shall also see whether alteration of fatty acids in cancer cell membrane lipids increases the cells' susceptibility to chemo and immuno-therapy and whether well-defined changes in cancer cell membranes alter the tumor-induced metabolic aberrations in the host. Finally, we shall study whether and how controlled alteration of the membrane fatty acid composition in culture or in vivo (peritoneal isolation) will alter pathways and rates of lipid and carbohydrate metabolism in cancers. In the course of testing these several hypotheses, we shall continue our efforts to develop radioactive tracer techniques, and multicompartmental approaches for studying complex metabolic interactions between tumor and host tissues in vivo.

BIBLIOGRAPHIC REFERENCES: Kannan, R., M. Ookhtens and N. Baker: Compartmental analysis of linoleate and palmitate turnover in a murine carcinoma. Cancer Res. 40:2447-2454, 1980. Kannan, R., I. Lyon and N. Baker: Dietary control of lipogenesis in vivo in host tissues and tumor of mice bearing Ehrlich ascites carcinoma. Cancer Res. 40, December, 1980, in press.

545. CELL TO CELL LIPID TRANSFER BETWEEN CELLS ON CONTACT

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Although the ability of cells to exchange and share phospholipids via serum lipoproteins is a well documented phenomenon, the intriguing possibility that direct cell to cell lipid exchange can occur as a result of contact between cells has not been fully explored. The general aim of this proposed research is to explore the conditions under which cell contact mediated lipid exchange occurs and to study its molecular basis.

Fluorescent lipids will be introduced into the outer leaflet of the plasma membranes of Chinese hamster V79 fibroblasts by exploiting the one for one lipid exchange between liposomes and cells recently documented in the sponsor's laboratory. Two different experiments will be performed to determine the transfer of fluorescently labelled phospholipids between cells. In the first, labelled cells will be mixed in suspension with unlabelled cells containing ingested latex beads. At timed intervals, aliquots will be removed and observed under the fluorescence microscope for the appearance of label on the originally unlabelled cells. In a similar experiment, labelled cells will be plated onto monolayer cultures of unlabelled cells containing latex beads. The spreading cells will be monitored periodically using Nomarski optics for the presence of fine processes and contacts between cells and by fluorescence for the transfer of label between cells. The role of temperature, divalent cation, and serum components on this process will be investigated. In addition we will investigate whether cell to cell lipid transfer can occur between different homologous and heterologous cell types.

546. MODIFICATION OF TUMOR CELL BEHAVIOR BY LIPOSOMES

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The uptake of liposomes of defined composition by cells in vivo and in vitro provides a useful experimental method for modifying cellular composition and for introducing biologically active materials into different compartments of the cell. Fusion of liposomes with the plasma membrane and/or exchange of components between the plasma membrane and liposomes adsorbed to the cell surface will be used to introduce specific phospholipids, glycolipids and cholesterol into the plasma membrane of normal and neoplastic cells in vitro to investigate the role of these components in the expression of specific cell surface properties and their effect on cell growth behavior in vitro and in vivo. In other, similar experiments 'membrane-vesicles' isolated from the plasma membrane of tumor cells will be fused with various acceptor cells. This fusion will permit transfer of plasma membrane proteins and glycoproteins between neoplastic cells exhibiting differing invasive and metastatic behavior so that the contribution of particular membrane proteins and glycoproteins in determining specific behavioral characteristics may be identified. These experiments will provide information on how changes in plasma membrane composition and cell surface organization in neoplastic cells are related to the altered behavioral properties displayed by these cells. Experiments are also proposed to evaluate the feasibility of using liposomes containing encapsulated lymphokines as a method for activating macrophage populations in tumor-bearing animals to augment host defense against tumor cells.

BIBLIOGRAPHIC REFERENCES: Fidler, I.J., Roblin, R.O., and Poste, G. (1978) The in vitro tumoricidal properties of activated macrophages against transformed lines with temperature dependent transformed characteristics. Cell Immunol., in press. Poste, G., Porter, C.W. and Papahadjopoulos, D. (1978) Identification of a potential artifact in the use of electron microscope autoradiography to localize saturated phospholipids in cells. Biochim. Biophys. Acta., in press.

547. LIPID METABOLISM OF LEUKOCYTES

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This proposal will examine the question of whether changes in membrane lipids are associated with an alteration in transport and susceptibility to chemotherapeutic agents. We have previously shown, by the use of experimental diets, that it is possible to alter the fatty acid composition of the membrane phospholipids of the L1210 murine leukemia. Using this model, we will examine the cellular transport of various anti-neoplastic agents including agents which are transported by carrier-mediated active transport, facilitated diffusion, and simple diffusion. In addition, we will examine the survival of mice carrying the L1210 leukemia treated with anti-neoplastic agents. For these studies, we will compare animals which are on the various experimental diets in order to assess the effects of the membrane lipid alteration.

548. PHYSICAL ANALYSIS OF LIPID-ALTERED TUMOR CELL MEMBRANES

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The effect of altering membrane fluidity on the ability of lymphocytes to undergo immune reactions will be explored. Membrane viscosity will be altered by precise control of the fatty acid composition of plasma membrane phosphatides. Composition can be altered both in terms of chain length and degree of saturation. Parameters that will be meausred include the binding and surface mobility of Con A; activation of T cells by Con A; activation of T cells by alloantigen in primary and secondary MLC; binding of cytotoxic effector T cells to target cells. The effect of altered membrane fluidity in the target cell (rather than the effector cell) on binding and cytolysis will also be determined. Finally, the possibility that some tumor cells can escape immune surveillance because of altered membrane fluidity will be examined by studying the ability of membrane-altered tumor cells to provoke a syngeneic immune response in vitro and in vivo.

549. RECIPROCAL CONTROL OF MEMBRANE PERMEABILITY OF TRANSFORMED CULTURES OF MOUSE CELL LINES BY EXTERNAL AND INTERNAL ATP

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Although maintenance of a selective permeability barrier is one of the essential functions of the cell surface, very little is known about the regulation of membrane permeability in animal cells. Recently we have found that treatment of spontaneously or virus-transformed mouse cells with external ATP causes a rapid and striking increase in membrane permeability which allows the entry of p-nitrophenylphosphate and the exit of nucleotides, phosphate esters and ions. None of these effects are

obtained with untransformed 3T3 cells. All these findings suggest that external ATP, when present, can regulate the permeability of the cell surface. We found that a variety of compounds known to reduce intracellular levels of ATP act synergistically with external ATP to cause a large increase in membrane permeability. The decrease in concentration of internal ATP was verified. Since eight compounds with quite different primary actions (uncouplers as well as inhibitors of respiration and energy transfer) were all active in greatly enhancing the effect of suboptimal external ATP, we felt that they function by decreasing internal ATP. This interpretation is supported by the fact that addition of glucose counteracts the synergistic effect of these substances on permeability, and by direct measurement of intracellular ATP levels. The efflux channel is sealed by bringing the transformed cultures to a neutral pH. Sealing requires energy and is prevented by the presence of inhibitors of energy metabolism.

550. PENETRATION OF MACROMOLECULES INTO MAMMALIAN CELLS

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Our research is devoted to the study of a fundamental membrane function of mammalian cells, namely the transport of macromolecules at the cell surface. This transport can be receptor-mediated or can occur in the absence of specific membrane receptors, in which case it is generally much less efficient. While our research encompasses both types of transport, our leading current interest is in procedures that will enhance the latter, i.e., that will facilitate the cellular uptake of maromolecules that are ordinarily only poorly transported. We have devised a procedure that dramatically increases the cellular uptake of such molecules (e.g., human serum albumin (HSA), horseradish peroxidase (HRP)) into cultured established lines of fibroblasts, sarcomas and carcinomas. The procedure consists of conjugating to the protein a fragment of poly(lysine). When poly(lys) of 6700 MW is conjugated to HRP at a ratio of approximately 1:1, the cellular uptake of active enzyme is increased by a factor of 400 (PNAS 75, 1872-76, 1978). This striking finding has raised a number of fundamental questions which have formed the basis of the current year's research and which will set goal for the coming year. It was shown in particular that poly(L-lysine) is avidly taken up by cells and that it can serve as carrier not only for proteins but for drugs (PNAS 75, 3867-70, 1978).

551. MEMBRANE PERMEATION AND ITS MODE OF ACTION

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Previous studies in the laboratory (Middleton, Journal of Membrane Biology, 1975, 20, 347; 1977, 34, 93; 1979, 47, 129) have indicated that hydrodynamic models of porous membrane transport, based on the Stokes-Einstein radius of a molecule, cannot realistically describe the passage of an asymmetric molecule through a membrane, even when the milieu fluid is water. Current studies have shown that hydrodynamic models are even more inappropriate when the molecule filters in the presence of other solutes. It is hoped to publish a paper on this matter shortly.

552. CARCINOGENESIS/MEMBRANE ENZYME AND PERMEABILITY MUTANTS

Hochstadt, J., New York University, School of Medicine, Dept. of Microbiology, *100 Washington Sq. E., New York, New York*, 10003, United States of America

Regulation of transport of nucleic acid precursors is well correlated with growth control phenomena in both normal and oncogenically transformed animal cell systems. In order to study the precise biochemical mechanisms of transport, including the enzymology and metabolic pathways, in vitro, mutants deficient in enzymes for the initial purine bases and nucleosides will be obtained. Three new classes of animal cell mutants which lack either purine nucleoside phosphorylase, adenosine deaminase, or 5' nucleotidase will be selected and analyzed for regulation, pathway and mechanism of transport in order to better elucidate the structure and function of cancer cells and normal cells.

Mutants for pleiotropic resistance to drugs involving a permeability change will be investigated for specific changes in transport systems and susceptibility to chemical carcinogenesis.

553. SURFACE LABELING OF MITOCHONDRIAL INNER MEMBRANE

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Many biological activities are determined by the structure of related biomembranes. In oxidative phosphorylation, the coupling mechanism is believed to be regulated by the structure of mitochondrial inner membrane. The present knowledge of the topography of this membrane is mainly derived from electron micrograms and biochemical properties assays of right-side-out and wrong-side-out vesicles. It is assumed that the membrane of digitonin vesicles has the same orientation as in the intact mitochondria. In the sonicated vesicles, however, the membrane has inverted orientation. This project outlines procedures using fluorescence energy transfer to establish the correct orientation of inner membrane in those vesicles. After these assignments, those proteins with -NH2 and/or tyrosine residues exposed to aqueous media will be identified by using SDS-acrylamide gel electrophoresis, amino acid composition analysis, and sequence determination. The understanding of the structure of mitochondrial inner membrane will certainly facilitate the elucidation of the molecular mechanism of coupling in oxidative phosphorylation. In addition, these methods should also be applicable to the investigations of the surface changes of cell membranes in neoplastic transformation and the probable membrane structure changes of mitochondria in alcoholic liver which exhibits abnormal ultrastructures.

554. MEMBRANE PATHOLOGY IN CARCINOGENESIS

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Significant changes in the cyclic AMP-dependent phosphorylation of specific plasma membrane proteins associated with malignant transformation have recently been identified in my laboratory and we have shown that aberrant activity of an endogenous plasma membrane cyclic AMP-dependent protein kinase is responsible for these changes. We have also identified differences in the cyclic AMP-dependent phosphorylation of plasma membrane proteins in differentiated and undifferentiated cells and have shown that modulation of cyclic AMP-dependent plasma membrane protein phosphorylation occurs during the G1 phase of the cell cycle in non-transformed cells. Studies proposed in this grant are designed to test the hypothesis that specific plasma membrane phosphoproteins influence control of cell growth in nontransformed cells but not in transformed cells. Experiments will be performed to characterize the endogenous cyclic AMP-dependent phosphorylation system in a variety of cell systems and to identify the biochemical basis for the defect in the phosphorylation system in transformed cells; both the protein kinase and its plasma membrane substrate will be studied. We will also examine the mechanisms for regulation of this phosphorylation system and we will attempt to identify the function(s) of the plasma membrane phosphoproteins especially their role in control of DNA synthesis and cell cycle kinetics.

BIBLIOGRAPHIC REFERENCES: Scott, R.E.: Aberrant cell cycle-cyclic AMP-dependent plasma membrane phosphorylation in transformed cells. J. Supramol. Struct., suppl. 3:202, 1979. Scott, R.E.: Modulation in cyclic AMP-dependent plasma membrane phosphorylation during the cell cycle and cell differentiation is defective in transformed cells. Fed. Proc., 38:919, 1979.

555. PHOSPHORYLATION OF HUMAN ERYTHROCYTE MEMBRANE PROTEINS (ALSO MICE)

Fairbanks, G., Worcester Foundation for Experimental Biology Inc., *222 Maple Ave., Shrewsbury, Massachusetts*, 01545, United States of America

This is part of a broader project. A summary of this subproject is not available.

556. STUDIES ON CELL SURFACE-BASEMENT MEMBRANE INTERACTIONS

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It is our aim to study the biochemical aspects of cell surface-basement membrane interactions. Analogous to cell-cell interactions and cell-circulating macromolecule interactions it appears likely that specific regions of the basement membrane and cell surface are involved in the attachment of cells to basement membrane. We plan to develop an assay using radiolabeled cells of a type which produces and rests on basement membrane, and basement membrane or basement membrane subunits attached to an insoluble surface. Utilizing this system, under a defined set of conditions we could monitor the attachment of cells to basement membrane by radioisotopic counting. The effect of mediating molecules (e.g., fibronectin) and inhibitors and/or competitors (e.g., peptides, glycopeptides, oligosaccharides, etc.) will be determined. Modification, either chemical or enzymatic, will be used to elucidate which components (e.g., native basement membrane, intact cells and fibronectin) are involved in the adhesive process. Eventually, we hope to identify and isolate the chemical determinants responsible for the adhesive phenomenon.

557. THE SYNTHESIS AND INTERACTION OF COMPONENTS MEDIATING SPECIFIC EMBRYONIC CELL ADHESION

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The mechanisms which mediate the formation and stability of intercellular attachments are critical to understanding both normal and aberrant developmental phenomena. The relative affinity of one cell for another is presumed to change during development so that cells form a continuum of differing architectural patterns with their neighbors, culminating in morphology characteristic for a particular organ or tissue. In addition changes in the adhesive capacity of neoplastic cells may be related to their ability to metastasize and invade surrounding tissue. Characterization of the surface structures involved in the formation of adhesions, their synthesis and turnover in the embryonic chick neural retina are the objectives of this research.

558. ALTERED CELL ADHESIVENESS IN VIVO - MEMBRANE CHANGES

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The membrane changes involved in the developmentally programmed decrease in cell adhesiveness that accompanies sea urchin embryo primary mesenchyme formation are being subjected to continued biochemical analysis. Specific projects in progress include: 1. Micromere isolation and differentiation of primary mesenchyme in vitro. 2. Analysis of a number of cellular events that occur during reaggregation of isolated micromeres. 3. Lectin agglutinability of developing primary mesenchyme cells. 4. Identification of lectin receptors on developing primary mesenchyme cells. 5. Galactose oxidase labeling of intact developing primary mesenchyme cells.

BIBLIOGRAPHIC REFERENCES: Turner, R.S., Jr. (1978) Sponge Cell Adhesions in Specificity of Embryological Interactions, ed. D. Garrod, 199-234 London: Chapman and Hall, Ltd.

559. PLASMA MEMBRANE AND METASTASIS IN RAT MAMMARY CANCER

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The proposed research is a study of the relationship between mammary tumor cell surface properties and the tumorigenicity and metastasis of the cells in a syngeneic host. Several tumor cell variant subpopulations have been isolated from the transplantable 13762 rat mammary adenocarcinoma by cloning and selection in vitro. The metastatic variants will be characterized in vivo by analysis of tumor growth at subcutaneous injection sites, short-term cell survival and organ selectivity of tumor cell arrest following injection of (125I) iododeoxyuridine-labeled cells, and patterns of long-term metastatic growth. Characterization in vitro will emphasize aspects of cell adhesiveness, to be defined by measurements of adhesion to substratum, self-aggregation, and attachment to monolayers of rat fibroblasts and endothelial cells. In biochemical studies of cell surface macromolecular composition and organization, glycoproteins, proteoglycans, antigens, and lectin-binding proteins will be identified by radiolabeling, enzymatic dissection, immunoprecipitation, and specific ligand binding; these components will be partially characterized by gel electrophoresis, and their patterns of organization will be determined using protein cross-linking reagents. The kinetics and selectivity of tumor cell surface shedding will be studied in vitro. Several variants differing in metastatic activity will be examined, so that results of biochemical analysis and in vitro characterization can be correlated with patterns of mammary tumor cell behavior in vivo.

560. CELL SURFACES AND GROWTH OF PANCREATIC CARCINOMA

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The goal of this project is to characterize membrane surface properties of pancreatic acinar carcinoma cells, and relate surface properties to growth of the acinar carcinoma. A mechanical fragmentation procedure has been developed for examination of the carcinoma in vitro. The fragments demonstrate normal rates and patterns of protein and DNA synthesis when maintained in vitro for up to 5 hours. The fragments were responsive to the secretagogue carbamylcholine between 10 to the minus 8th power and 10 to the minus 3rd power M, suggesting good preservation of membrane receptors for secretagogues during tumor fragmentation. However, the carcinoma fragments demonstrated an apparent low rate of carbamylcholine-induced protein secretion as compared to normal pancreas in vitro. We suggest that the apparent low rate of protein secretion reflects functional immaturity of the acinar carcinoma. Future work will focus on further characterization of membrane receptors, protein secretion, and cell proliferation in the carcinoma.

BIBLIOGRAPHIC REFERENCES: Warren, J.R., Becich, M.J., and Reddy, J.K. Macromolecular synthesis and secretion by fragments of pancreatic acinar cell carcinoma. Federation Proceedings 39: 639 (1980).

561. BIOCHEMICAL AND GENETIC STUDIES OF THE SURFACE MEMBRANES

Clayton, B., Wistar Inst. of Anatomy & Biology, *36th & Spruce Sts., Philadelphia, Pennsylvania*, 19104, United States of America

This is part of a broader project. A summary of this subproject is not available.

VIII. STUDIES ON THE METABOLISM OF CALCIUM AND OTHER ELEMENTS IN NORMAL AND NEOPLASTIC CELLS

562. CA 2 ION METABOLISM IN NORMAL AND TRANSFORMED LYMPHOCYTES

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Our long-term objective is the characterization of Ca 2 ion metabolism in normal and neoplastic cells. To attain this objective we propose (1) a detailed quantitative description of Ca 2 ion binding and uptake by normal and GD 248 leukemic hamster lymphocytes; (2) a qualitative description of Ca 2 ion - membrane interaction; and (3) a study of the effect of Ca2 ion on different metabolic functions of these cells and on membrane structure. A quantitative analysis of Ca 2 ion uptake by the cells will be achieved with standard Millipore-filtration methods. The qualitative details will be obtained in experiments with purified membrane fractions and intact cells employing fluorescent-lanthanides as Ca 2 ion probes and enzyme probes such as neuraminadase, phosphatidylserine decarboxylase. We will examine the

functional role of Ca 2 ion in the regulation of glycolysis and respiration using standard biochemical techniques. The effect of Ca 2 ion on membrane structure will be analyzed with hydraulic and lipophilic photoactivated cross-linkers in conjunction with SDS-polyacrylamide electrophoresis to evaluate changes in protein-protein neighbor relationships. Ca 2 ion induced phase transitions in membrane proteins and lipids will be monitored by Raman spectroscopy. To relate the cell biology results obtained with intact cells with the biochemical characterization obtained with isolated membrane fractions we will employ the cyanine dye fluorescence method to monitor the membrane potential of intact cells, mitochondria and sealed plasma membrane vesicles under different physiological conditions, e.g., Ca 2 ion concentration, pH.

BIBLIOGRAPHIC REFERENCES: Mikkelsen, R.B., Schmidt-Ullrich, R., and Wallach, D.F. H. (1979) Federation Proceedings 38, 1431. Mikkelsen, R.B. and Schmidt-Ullrich, R. (1979) J. Biol. Chem., in press.

563. MOLECULAR BASIS OF CALCIUM ACTION IN CELL PROCESSES

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The objective of this proposed project is to understand the molecular mechanism(s) whereby calcium exerts its effects on and regulates cell processes. Calcium exerts its biological effects via interaction with calcium modulated proteins. Calmodulin, a calcium modulated protein, regulates in a calcium dependent manner a number of enzyme activities. This suggests that calmodulin may be a pleiotropic regulator of metabolic and mechanochemical processes in the cell. Calmodulin regulated enzymes will be isolated using a combination a affinity chromatography and classical protein chemistry methodologies. Calmodulin and calmodulin regulated enzymes will be localized in normal and virus-transformed fibroblasts. The isolation, characterization and subcellular localization of a calcium modulated protein and of the enzymes which it regulates will contribute to our knowledge of how calcium regulates normal cell processes and might provide new insights into the molecular mechanism(s) of cell transformation.

BIBLIOGRAPHIC REFERENCES: Watterson, D.M., Iverson, D.B. and Van Eldik, L.J. 1980. Rapid separation and quantitation of 3'-nucleotides in phosphodiesterase reaction mixtures using high-performance liquid chromatography. J. Biochem. Biophys. Methods, in press. Van Eldik, L.J. and Watterson, D.M. 1979. Characterization of a calcium modulated protein from transformed chicken fibroblasts. J. Biol. Chem. 254:10250-10255.

564. THE BIOCHEMISTRY OF CELLULAR TRANSFORMATION

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The divalent cation, Ca2 ion, serves as a regulatory signal through which eukaryotic cells perceive and respond to both internal and external stimuli. A single protein, calmodulin (CaM), is the primary intracellular receptor for Ca2 ion in most tissues where it serves as a Ca-dependent activator of numerous enzyme systems. Various lines of evidence suggest that the deregulation of animal cell growth upon cellular transformation may involve the perturbation of Ca-CaM regulated enzyme systems. Clearly, the pleiotropic effects seen on cellular transformation suggest that major cascade regulation systems, possibly those regulated by CaM, are perturbed by the transforming principle. Studies described here to test this possibility include: 1) Determining the presence and relative levels of CaM and CaM binding proteins, particularly those of known enzymatic activity, in normal chick embryo fibroblasts (CEF) and CEF infected with wild-type (wt) and transformation deficient (td) mutants of Avian sarcoma viruses (ASV) under various conditions of growth; 2) Examining the phosphorylation state of CaM binding proteins in normal and transformed cells and testing them as potential src kinase substrates both in vivo and in vitro using O-tyrosylphosphorylation as an assay for src kinase specific phosphorylation; 3) Testing whether or not, pp60src, is regulated either directly by a Ca-dependent interaction with CaM or indirectly through CaM-dependent protein kinases; 4) Examining by HPLC the in vivo pool levels of nucleotides, NAD, and NADP in normal and virally transformed CEF under a variety of growth conditions to test for possible transformation dependent perturbation and/or CaM-dependent regulation; 5) Detailed analyses of NAD kinase in normal and transformed CEF for possible CaM dependent regulation and transformation dependent alteration; and 6) Examination of other enzymes of nucleotide biosynthesis, such as ribonucleotide reductase for regulation by CaM in normal and transformed CEF.

565. ORGAN CULTURE STUDIES OF SYNTHESIS AND FUNCTION OF CABP

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OBJECTIVE: Elucidate the mechanism of action of vitamin D(3), and related sterols, in the induction of calcium binding protein (CaBP), the nature of the vitamin D, cyclic AMP, CaBP, Ca interaction, and the role(s) of CaBP and cyclic AMP, and other factors, in intestinal calcium absorption. Determine the feasibility of the use of an organ culture system in the study of other aspects of intestinal physiology, e.g., transport of nutrients other than calcium, drug transport, glycoprotein synthesis and chemical carcinogenesis.

APPROACH: Several variations of a novel organ culture technique, utilizing embryonic chick duodenum, have been developed. The cultured duodenum responds to vitamin D(3) and related sterols by an early rise in cyclic AMP concentration followed by de novo synthesis of a specific CaBP and enhanced transmucosal transport of calcium. The system, therfore, closely mimics what may be seen in vivo but has the obvious advantage of being under strictly defined conditions completely isolated from systemic influences.

PROGRESS: Experiments have continued using the embryonic chick duodenum in organ culture. During the past year the following has been accomplished: First, studies of the biological potency of vitamin D-analogs, particularly fluoro derivatives, have been undertaken using as a biological endpoint, the induction of a specific, intestinal calcium-binding protein (CaBP) in vitro. Substitution of the 25-C atom with N reduces biopotency to about 1/500,000th that of 1 Alpha, 25-(OH)(2)-D(3). Of the fluoro analogs, 1 Alpha-F-D(3) was unexpectedly potent, about 1/2 as active as 1 Alpha, 25-(OH)(2)-D(3), whereas vitamin D(3) itself is only 1/1000th as active. Similarly, 1 Alpha, 25-F(2)-D(3) was 50 times as active as D(3). All of the 25-fluoro analogs were less potent than their non-fluorinated counterparts. Of greatest interest was the finding that 24,24-F(2)-1 Alpha, 25-(OH)(2)-D(3) was about 4 times as potent as the hitherto most active vitamin D(3) analog, 1 Alpha, 25-(OH)(2)-D(3). (Text Abridged.)

566. CALCIUM METABOLISM IN MEDULLARY CARCINOMA OR PHEOCHROMOCYTOMA (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

567. STRUCTURE OF FERRITIN AND ITS ROLE IN IRON METABOLISM

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The subunit composition and assembly mechanisms of ferritin from various sources will be examined. Aspects of primary structure, conformation and molecular interactions will be probed by physical and chemical means, in an effort to gain insight into the mechanisms by which the protein mediates iron deposition and mobilization in mammalian cells. Aspects of microheterogeneity of different ferritins will be related to subunit heterogeneity. The two classes of subunits found in most ferritins (H and L types) will be purified to homogeneity, and reassembled to form discrete apoferritin moieties. Iron loading functions, and other properties of these apoferritins will be studied. Specific antisera (H or L) will be prepared to determine

levels of different species of ferritin in tissues and sera. Biosynthesis studies and mechanisms of differential regulation of the two subunits in response to iron will be investigated in detail.

BIBLIOGRAPHIC REFERENCES: P. Aisen and I. Listowsky (1980) Ferritin. Ann Rev. Biochem. 49, 357-393. M.M. Bhargava, N. Ohmi, I. Listowsky and I.M. Arias (1980) Structural Catalytic Binding and Immunological Properties Associated with Each of the Two Subunits of Rat Liver Ligandin. J. Biol. Chem. 255, 718-723.

568. IRON METABOLISM IN CANCER - SPECIAL REFERENCE TO LYMPHOMAS AND LEUKEMIAS

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The various biochemical factors involved in iron metabolism, such as iron, iron binding capacity, copper, magnesium, ceruloplasmin, ferritin, etc. have been estimated by biochemical methods in the sera of 300 patients with lymphomas and 200 patients with various types of other cancers. Other parameters such as zinc, manganese, vitamin B12 and folic acid have also been included in this study.

Studies on Experimental Lymphomas: Dalton's lymphoma cell lines were used to induce ascites as well as solid tumors by the 'Air-Pouch' technique in Swiss mice. Control mice either contained the air pouch (no tumor) or were used as such. Ten pairs of animals were used in this experiment and sacrificed and the iron content of serum and the following organs - liver, spleen and kidneys - was estimated after acid digestion. The iron content in micrograms g/gm wt. of tissues in livers of the ascites and solid tumor group were lower compared to the controls, whereas in the spleen the iron level was significantly higher in the ascities and solid tumor group. Kidney level showed a slight variation. Further work on the biochemical mechanism involved in these alterations is in progress.

569. COPPER ABSORPTION AND CERULOPLASMIN IN CANCER

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The objective of the proposed research is to follow up on our interesting observation that copper absorption is enhanced in tumor bearing rats. This increase occurs in conjunction with other aberrations of copper metabolism in cancer, especially in elevated concentration and enhanced synthesis of the circulating copper protein, ceruloplasmin. Since so little is known about the basic mechanism of intestinal copper absorption and its regulation we will first study this process thoroughly in normal rats, and then make comparison with tumor bearing animals. The studies proposed involve in vivo and in vitro studies using 67Cu and 64Cu, following the uptake and distribution of copper within the mucosa, and its further transfer across the serosal surface. The influence of copper status and hormonal factors in the process of absorption will also be examined. It is expected that such studies will provide much basic information on the mechanism and regulation of copper absorption and explain the increased absorption which occurs in animals with tumors.

570. MODELING THEORY AND APPLICATIONS

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Analysis of various kinetic and metabolic systems by the use of mathematical models carried out with other investigators. These include long range zinc studies in man (Babcock, Foster), extension of the deconvolution technique to time dependent systems, etc.

BIBLIOGRAPHIC REFERENCES: Babcock, A.K., Garvey, T.Q., III, and Berman, M.: A mathematical model for membrane transport of amino acid and Na ion in vesicles. J. Biol. Chem. 49: 157-169, 1979. Foster, D.M., Aamodt, R.L., Henkin, R.I. and Berman, M.: Zinc metabolism in humans: A kinetic model. Am. J. Physiology. 237: 340-349, 1979.

571. MODIFICATION OF TUMOR CELLS AND TUMOR SYSTEMS

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Our aim is to determine whether the rate of growth of non-tumor cells is limited or controlled, under physiological conditions, by the levels of intracellular monovalent cations or the activity of the Na/K ATPase pump system. We would also like to find out if tumor cells have escaped from such a regulatory system.

BIBLIOGRAPHIC REFERENCES: Ledbetter, M.L.S., and Lubin, M. Interference of Ouabain with the Demonstration of Cell-to-Cell Potassium Transfer. J. Cell Biology 79 (2,Pt.2):80a (1978). Ledbetter, M.L.S. and Lubin, M. Transfer of Potassium: A New Measure of Cell-Cell Coupling. J. Cell Biology 80:150-165 (1979).

572. NUTRITIONAL AND BIOMEDICAL INTERACTIONS OF SELENIUM

Oldfield, J. E., Weswig, P. H., Schmitz, J. A., Oregon State Higher Education System, Oregon State University, Agricultural Experiment Station, Dept. of Animal Science, *126 Agriculture Hall, Corvallis, Oregon,* 97331, United States of America

OBJECTIVE: Determine effects of natural and synthetic substances on Se metabolism. Investigate Se deficiency in species other than cattle and sheep. Study effects of Se on reproduction anticarcinogenicity and vascularization. Investigate synthesis of Se-binding proteins. Identify factors responsible for calcification in white muscle disease. Compare muscle lysosomes from normal and Se deficient animals.

APPROACH: Se deficiency will be induced in lambs by feeding pregnant ewes forage imported from Se-deficient soil areas. Blood and other tissues in normal and deficient animals will be examined. Effects of organic and inorganic constituents of the feed on Se status will be examined. Similar studies will be done on lab animals and other species, using torula diets to induce deficiency.

PROGRESS: Interest centered on a specific source of alfalfa hay which when fed to pregnant ewes under field conditions, caused white muscle disease (WMD) in lambs even though selenium and vitamin E were given at recommended levels. In a controlled study, pregnant ewes were fed this hay plus oats (22 ewes) or this hay plus oats supplemented with selenium to equal 0.1 ppm on total intake basis (16 ewes). All but 3 lambs of a total of 31 born in the untreated group died before 6 weeks of age. The remaining 3 lambs eventually died at ages of 8 to 10 weeks. All lambs which died had WMD. In contrast, 12 of the 20 lambs born in the selenium treated lot died before 6 weeks of age, showing symptoms of WMD. Thus, the accepted level of supplementary selenium did not give protection against WMD with this hay. As expected, the blood selenium content and erythrocyte glutathione peroxidase activity were higher in lambs from the selenium supplemented than deficient group. Since calcium accumulates in skeletal muscle of WMD lambs, the calcium transport membranes in these affected lambs were investigated. The membranes from muscle of WMD lambs showed little ability to transport calcium whereas membranes from selenium supplemented lambs were very active in calcium transport. Calcium dependent ATPase was lower whereas the total ATPase was higher in membranes from WMD lambs as compared to normals. One reason for this accumulation of calcium may be that these membranes have lost their ability to transport the element.

573. INVESTIGATION INTO SIGNIFICANCE OF TRACE ELEMENT CONCENTRATIONS AND THEIR CHANGES IN DIAGNOSIS AND FOLLOW-UP OF MALIGNANCIES BY PIXE

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There is much information on the changes of trace elements, notably trace metals such as Zn, Cu, Cr, Mn, etc., in human biological fluids and tissue specimens in malignant diseases. However, the reports are often contradictory and inconsistent, and in most cases clinical advantages of trace element determinations in malignancies have not been identified. The

objective of this study is to investigate trace metal concentrations and their changes during treatment of malignant diseases using a multi-element method, particle-induced X-ray emission analysis (PIXE), with which it is possible to obtain simultaneously several (6-10) trace metals from a biological specimen (both fluids and tissues) with minimal manipulation of the samples and with sensitivity of 0.1-0.01 ppm. By computer analysis not only changes in concentrations of isolated trace metals but also in their ratios can be investigated and their significance stated.

Trace element analysis will be made using PIXE analysis in the Cyclotron Laboratory of Abo Akademi (J. Dahlbacka). Atomic absorption spectrophotometry and neutron activation analysis will be used as complementary methods. The patient material will be analyzed by Prof. E. Nordman (Department of Oncology, University Hospital) and by Dr. A. Rajamaki (hematological cases).

Patients with breast cancer, gastrointestinal cancers, leukemias and Hodgkin's disease will be included in the material. Specimens of tissue are obtained from these patients which are operated on. From patients with hematological malignancies white blood cells are isolated for analysis. Serum samples are obtained from all patients and in selected cases the daily urinary excretion of trace metals is measured. Trace metals determined include Zn, Cu, Mn, Cr, Ni, Sr, Br, Mg, Ca.

574. THE BIOLOGICAL FUNCTION OF METALS

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This application details approaches to the characterization of metalloenzymes from a number of species, e.g., bovine, equine and Bacillus (carboxypeptidase, alcohol dehydrogenase, thermolysin) and others, both to discern their mechanisms of action and to uncover general laws concerning the coordination of metals in enzymes as it pertains to their functions. This program in general biochemistry will encompass studies of the possible functional role of horse metallothionein, a Cd and/or Zn containing protein first isolated in this laboratory 20 years ago.

These studies will be carried out side by side with those on analogous enzymes and proteins isolated by us from man, e.g., human carboxypeptidase, alcohol dehydrogenase, metallothionein and zinc proteins from human serum, on the metabolism of human leukemic cells and their constituent zinc proteins. These investigations of human biochemistry will extend those in general biochemistry and serve to investigate the roles of metalloenzymes in pathology.

BIBLIOGRAPHIC REFERENCES: Thompson, J.S., Gehring, H. and Vallee, B.L. Structure and Function of Carboxypeptidase A alpha in Supercooled Water. Proc. Natl. Acad. Sci. U.S.A. 77, 132-136, 1980. Scheule, R.K., Van Wart, H.E., Vallee, B.L., and Scheraga, H.A., Resonance Raman Spectroscopy of Arsanilazocarboxypeptidase A: Conformational Equilibria in Solution and Crystal Phases. Biochemistry, 19, 759, (1980).

575. SERUM-CALCITONIN LOWERING EFFECT OF MG IN THYROID CARCINOMA (HUMANS, MONKEYS)

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This is part of a broader project. A summary of this subproject is not available.

IX. VITAMINS AND OTHER TOPICS IN CANCER-RELATED BIOCHEMISTRY

576. CASE-CONTROL STUDY OF LUNG CANCER AND DIETARY VITAMIN A

Kolonel, L. N., Hinds, M. W., Nomura, A. M., Hankin, J. H., Lee, J., University of Hawaii System, Manoa Campus, Pacific Biomedical Research Center, Epidemiology Program, *2538 The Mall, Honolulu, Hawaii*, 96822, United States of America

Over a period of 3 years, 500 lung cancer patients and 1,000 age and sex-matched neighborhood controls will be interviewed. Men and women of Caucasian, Japanese, Chines, Filipino, and Hawaiian/part-Hawaiian ethnic groups will be included. Trained interviewers will administer a questionnaire in the homes of cases and controls. The questionnaire will include both frequency and quantitative information on approximately 75 dietary items as well as smoking and occupational histories. The dietary items have been chosen to account for at least 85 percent of vitamin A intake for each ethnic group based on previously collected 3-day diet records. The object of the study will be to test the hypothesis of a negative relationship between lung cancer risk and dietary vitamin A.

BIBLIOGRAPHIC REFERENCES: Kolonel, L.N. Smoking and Drinking Patterns among Five Ethnic Groups in Hawaii. In: Second Symposium on Epidemiology and Cancer Registries in the Pacific Basin, Maui, January, 1978, Natl. Cancer Inst. Monograph 53:81-87, 1979. Hinds, M.W. and Kolonel, L.N. Malignant melanoma of the skin in Hawaii, 1960-1977. Cancer 45:811-817, 1980.

577. CANCER AND VITAMIN A

Ong, D. E., Page, D. L., Vanderbilt University, School of Medicine, Dept. of Biochemistry, *21st Ave. S. & Garland, Nashville, Tennessee*, 37232, United States of America

The proposed research is designed to contribute to the understanding of the prophylactic and therapeutic action of vitamin A and synthetic analogues on some neoplasms. Specific attention will be given to the cellular binding proteins for vitamin A, particularly from humans. The main objectives will be to develop antibodies against human binding proteins for radioimmunoassay and immunolocalization. These methods will be used to characterize human cancers and precancerous states. Techniques will be developed in model rat systems.

BIBLIOGRAPHIC REFERENCES: Lotan, R., Ong, D. E. and Chytil, F. Comparison of the Level of Cellular Retinoid-Binding Proteins and Susceptibility to Retinoid-Induced Growth Inhibition of Various Neoplastic Cell Lines J. Natl. Cancer Inst. 64, 1259-1262 (1980). Kylberg, H. K., Ong, D. E. and Chytil, F. Cellular Retinol-Binding Protein During Post-Natal Development of the Ram Small Intestine. Biol. of the Neonate, in press (1980).

578. ANTINEOPLASTIC ACTION OF VITAMIN A IN SKIN CANCER

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Natural and synthetic retinoids offer exciting possibilities as epithelial cancer-preventing and cancer-treatment agents. Although their antineoplastic potency has been demonstrated in numerous animal models, their usefulness in human malignancy is only now being ascertained. Furthermore, the mechanism(s) whereby retinoids influence epithelial differentiation remains unknown. In this proposal we will be assessing the subcellular basis for the retinoids' activity against cancer, focusing on changes in membrane structures, particularly gap junctions, in microfilaments, and in the basement membrane in human basal cell carcinomas treated with topical retinoids. In addition, we wish to ascertain by clinical testing if the retinoids are clinically useful for the therapy of established basal cell cancers. Preliminary results indicate that retinoids stimulate a proliferation in the size and number of gap junctions in basal cell cancer cell membranes. Since gap junctions are important for intercellular communication and cell regulation, this may represent one possible mechanism for the retinoid's antineoplastic activity.

BIBLIOGRAPHIC REFERENCES: Elias, P.M., Fritsch, P.: Erythema multiforme. In: Dermatology In General Medicine (ed. Fitzpatrick, T.B., et al) McGraw-Hill, 1979, in press. Elias, P.M., Fritsch, P.: Staphylococcal scalded skin syndrome. In: Dermatology In General Medicine (ed. Fitzpatrick, T.B., et al) McGraw-Hill, 1979, in press.

579. NUTRITIONAL ASPECTS OF VITAMIN A AND CANCER

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OBJECTIVE: Vitamin A and related compounds (retinoids) have been shown to have preventive and therapeutic effects on several types of tumors in the intact animal, to provent carcinogen-induced hyperplasia in organ culture, and to restore some functions of normalcy to transformed cells in culture. Vitamin A deficient animals are also more susceptible to carcinogens and may be more prone to development of spontaneous tumors. The overall objective is to study the anti-tumor effects of retinoids. Specifically, the proposal is to study the effects of retinoids on tumor cells in cultures; a. to develop an in vitro model for the study of the direct effects of retinoids on tumor cell proliferation, b. to study direct effects of retinoids on parameters of transformation, c. to resolve the discrepancy of results of retinoid treatment on tumors grown in vitro and in the animal, and, d. to study the mechanism of action of retinoids on DNA synthesis and control of proliferation of tumor cells.

BIBLIOGRAPHIC REFERENCES: Dion, L.D., and Gifford, G.E. Vitamin A-induced modulation of the transformed cell phenotype in vitro. In Modulation of Cellular Interactions by Vitamin A and Derivatives (retinoids), L.M. De Luca and S.S. Shapiro, editors. Ann. N.Y. Acad. Sci. 359:389-390, 1981. Ruff, M.R. and Gifford, G.E. Tumor necrosis factor. In Lymphokines vol. 2, E. Pick, editor. Pp. 235-272, 1981. Academic Press, N.Y.

580. METABOLISM AND TISSUE DISTRIBUTION STUDIES OF NATURAL AND SYNTHETIC RETINOIDS

Roberts, A. B., U.S. Dept. of Health & Human Services, Public Health Service, National Inst. of Health, National Cancer Inst., Laboratory of Chemoprevention, *9000 Rockville Pike, Bethesda, Maryland*, 20205, United States of America

The purpose of this project is to increase our understanding of the metabolism of retinoids. Such information must be had before a rational approach can be taken to the design of new retinoids with greater activity in the reversal of preneoplasia in epithelial tissues. The investigation is focused on a study of the in vitro metabolism of retinoic acid and synthetic retinoids in subcellular systems of target tissues as compared to non-target tissues.

BIBLIOGRAPHIC REFERENCES: Roberts, A.B., Nichols, M.D., Newton, D.L., and Sporn, M.B.: In vitro metabolism of retinoic acid in hamster intestine and liver. J. Biol. Chem., 254: 6296-6302, 1979. Roberts, A.B., Lamb, L.C., and Sporn, M.B.: Metabolism of all-trans-retinoic acid in hamster liver microsomes: oxidation of 4-hydroxy- to 4-keto-retinoic acid. Arch. Biochem. Biophys. 199: 374-383, 1980.

581. BIOCHEMICAL DIFFERENCES BETWEEN NORMAL AND LEUKEMIC WHITE CELLS

Huennekens, F. M., Suresh, M. R., Scripps Clinic & Research Foundation, Dept. of Biochemistry, *476 Prospect St., La Jolla, California*, 92037, United States of America

The goal of this program is to search for biochemical differences between normal and malignant mammalian cells at the molecular level and to exploit these differences in the synthesis or improved use of chemotherapeutic agents. Studies on folate-dependent enzyme systems and folate transport systems are focused upon their interaction with the folate antagonist methotrexate (MTX), the 4-amino-10-methyl analog of folate, which is used extensively in the treatment of leukemia and other neoplastic diseases. In particular, efforts are directed toward: (a) examination of the molecular targets (primary dihydrofolate reductase, and the 5-methyltetrahydrofolate transport system) that are attacked by MTX; (b) understanding how these drug-target interactions lead to inhibition of cell replication; and (c) determination of why these targets fail to respond to MTX in resistant cells. Specific projects include: (a) Adenine-pteridine transport system in L1210 cells; (b) 5-methyltetrahydrofolate/MTX transport system in L1210 cells; (c) structure-function relationships in L1210 dihydrofolate reductase; and (d) structure and mechanism of methionine synthetase.

582. CHARACTERIZATION OF THE METABOLISM OF CHEMOPREVENTION RETINOIDS

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No summary has been provided to the Smithsonian Science Information Exchange.

583. NEW GEOMETRIC ISOMERS OF VITAMIN A AND CANCER PREVENTION

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This is part of a broader project. A summary of this subproject is not available.

584. THE ROLE OF VITAMINS AND NUTRIENTS IN CELLULAR DEVELOPMENT

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OBJECTIVE: Initial emphasis will be on the role of vitamins in the central nervous system development with the following aspects being investigated: Development of cytoarchitectural alterations and myelination; correlation between nutritional status and morphological-biochemical changes; relationship between dietary levels of the nutrient and onset of symptoms resulting from either deficient or excessive amounts of the nutrient in the diet. Ancillary studies will include both biochemical and histological changes in skin, blood, milk, hair and various organs and the role of nutritional status in the etiology of neoplastic disease.

APPROACH: Feed ad libitum diets containing and adequate, deficient or excessive amount of a nutrient to weanling female rats from growth through lactation. Use a correlative biochemical-morphologic approach to study effects on the central nervous system and various organs of the progeny.

PROGRESS: Two projects are reported; a joint study with Professor A. Kirksey concerns a correlative morphological-biochemical approach to study the developing brain and spinal cord in vitamin B-6 deficient rats. Morphometic measurements showed decreased white matter area and number of large diameter axons in the cervical spinal cord of vitamin B-6 deficient rats compared to controls. These reductions correlated with low specific activity of the myelin marker enzyme, 2', 3'-cyclic nucleotide 3' phosphohydrolase, in the same region of the spinal cord. Regional distribution of pyridoxal phosphate (PLP), the active coenzyme form of vitamin B-6, as determined by a tyrosine decarboxylase apoenzyme radioassay, showed lower concentrations of PLP in all regions of the deficient CNS compared to the control. Vitamin A is emerging as a potential cancer chemopreventative agent. To investigate its effect on tumor establishment, growth and metastasis, rats were fed varying amounts of vitamin A prior to subcutaneous injection of either a metastatic or non-metastatic line of transplantable hepatocellular carcinomas derived from solid tumors induced by N-2-fluorenylacetamide. Tumors were fewer and appeared later under conditions of vitamin A excess. No metastases were observed in animals fed excess vitamin A; however, 60% had pulmonary metastases in the vitamin A deficient group and 75% in the vitamin A adequate group.

585. VITAMIN B6 AND HORMONE ACTION IN UTERI AND BREAST CANCER

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The response of normal and malignant estrogen target tissues to estradiol is determined by the concentration and retention time of nuclear receptor estrogen complexes (RE2). Vitamin B6 inhibits nuclear binding of RE2 by interfering with the cytoplasmic activation and nuclear retention reactions in vitro. It is the purpose of this study to further characterize these reactions and to determine if similar events can be observed in vivo using normal rat uterus and DMBA-induced mammary tumors. Furthermore, in vitro physico-chemical analysis of estrogen receptor and anti-estrogen receptor states in the cytoplasm and nuclear cell fractions will be carried out in the presence and absence of specific chemical probes.

586. VITAMIN B12

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This is part of a broader project. A summary of this subproject is not available.

587. VITAMIN D-PARATHYROID INTERACTIONS

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The direct effects of 1,25-dihydroxyvitamin D3 on chicken parathyroid gland function will be studied. The chicken will be used as an experimental animal because of the ease with which its vitamin D status can be controlled and the extensive work that has been done on vitamin D receptors in this system. Chick parathyroid hormone will be purified by conventional techniques and used to generate a specific antiserum. Utilizing the specific antiserum, I will study the direct effects of 1,25-dihydroxyvitamin D3 on parathyroid hormone synthesis and secretion. Both in vivo and in vitro approaches will be used in order to identify the locus or loci of steroid action in this tisssue. Possibilities to be explored are transcriptional control, translational control, post-translational processing and modulation of peptide secretion. Specific antiserum will be used for developing a radioimmunoassay to chick parathyroid hormone and for immunoprecipitating newly synthesized polypeptide chains. These two general methods, with appropriate experimental designs, will allow the measurement of the levels of the hormone, the levels of its mRNA, the rate of synthesis of the hormone, the post-transcriptional processing of the precursor form of the peptide to the secretory form and the rate of secretion of the hormone. These studies should allow me to identify and characterize the site of action of 1,25-dihydroxyvitamin D3 in the parathyroid glands and to broaden our understanding of the endocrine regulation of calcium homeostasis.

588. VITAMIN D RESISTANT RICKETS SECONDARY TO TUMORS (HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

589. EFFECT OF VITAMIN E AND LIPIDS ON TUMORIGENICITY

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Our major goal is to test the effect of dietary vitamin E on the tumorigenicity of established tumor cell lines. We have shown that the vitamin can decrease the tumorigenicity of transplanted tumor cells. This protection is dependent on the dose of tumor cells as well as the dose of vitamin E. The latter goes through an optimum and loses its protective ability at very elevated levels (5g/kg diet). We have shown that vitamin E enhances the response of murine spleen cells to respond to T cell mitogens: Con A and PHA. The ability to respond to these mitogens is taken as a measure of the potential to protect against tumors by cell mediated immunity. The involvement of the immune system in the protection produced by vitamin E was established by showing that protection was abrogated when the mice were exposed to sublethal irradiation (400R). It has been demonstrated in preliminary studies that vitamin E may enhance the antigenicity of the tumor cell as well as its ability to act as a target. We will explore the relationship of vitamin E, the immune system and tumorigenicity. With the aid of monoclonal antibodies to the tumor associated antigens, we will investigate whether vitamin E can increase the antigen display on the tumor cells, therby rendering it a better antigen and possibly a better target. The ability to act as a target may also reflect a cell's ability to repair membrane damage, specifically by increased membrane phospholipid synthesis (Schlager and Basos). Since our tumor cells are affected by the kind of lipid in the diet this is a problem we wish to investigate.

BIBLIOGRAPHIC REFERENCES: Corwin, L.M., and Shloss, J. 1980. Influence of vitamin E on the Mitogenic Response of Murine Lymphoid Cells. J. Nutr. 110: 916-923. Corwin, L.M., and Shloss, J. 1980. Role of Antioxidants on the Stimulation of the Mitogenic Response. J. Nutr. 110: 2497-2505.

590. REGULATION OF FOLATE POLYGLUTAMATE SYNTHESIS

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Folypoly-gamma-glutamate synthetase will be purified from a variety of bacterial and mammalian sources, and its substrate specificity and kinetic properties examined to determine whether they are responsible for the different types of pteroylopolyglutamates found in different cell lines. Pteroylpolyglutamate substrates and potential analog inhibitors of the synthetase will be synthesized by a solid phase procedure.

The effects of products of one-carbon metabolism, such as methionine, thymidine, and adenosine, on the folate requirements, the glutamate chain lengths of endogenous folates, and folypoly-gamma-glutamate synthetase levels will be examined in bacteria and a variety of tumorigenic and nontumorigenic mammalian cells. These studies will investigate whether one-carbon metabolism is controlled by varying the glutamate chain length of endogenous pteroylpolyglutamates and whether there are differences between normal and malignant cells in folate requirements and requirements for products of one-carbon metabolism. Inhibitors of folypoly-gamma-glutamate synthetase will be tested for their ability to cause cellular folate depletion by preventing the formation of pteroylpolyglutamates, the forms of vitamin that are selectively retained by tissues.

BIBLIOGRAPHIC REFERENCES: Brody, T., Shane, B., and Stokstad, E.L.R. (1979) Separation and Identification of Pteroylpolyglutamates by Polyacrylamide Gel Chromatography. Anal. Biochem. 92, 501-509. Shane, B., Brody, T., and Stokstad, E.L.R. (1979) Pteroylpolyglutamate Synthesis by Corynebacterium species. In: Chemistry and Biology of Pteridines, Kisriuk, R., and Brown, G.M., eds. pp. 341-346. Elsevier, New York.

591. BIOCHEMISTRY OF PTEROYLPOLYGLUTAMATES

Krumdieck, C. L., University of Alabama in Birmingham, School of Community & Allied Health, Dept. of Nutrition Sciences, *Tidwell Hall BOO6, University Station, Birmingham, Alabama*, 35294, United States of America

This is part of a broader project. A summary of this subproject is not available.

592. MOLECULAR ASPECTS OF ENERGY UTILIZATION, CELLULAR FUNCTION AND CARCINOGENESIS

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OBJECTIVE: To improve understanding of the role of free radicals and other paramagnetic species in cellular function with special reference to carcinogenesis.

APPROACH: Pulse radiolysis is being used to produce free radical and other biologically relevant species under conditions where their sections can be unambigously identified. In many cases this permits characterization of reactions which have been put forward speculatively to account for enzyme action. Among the topics being studied are the cytochrome P450 component of mixed function oxidases, vitamin B12 and lipid peroxidation. Increasing attention is being paid to micellar systems so as to identify characteristics imposed by an inhomogeneous environment.

PROGRESS: The addition of the first electron to the cytochrome P450 of Pseudomonas putida can be mimicked by one-electron reducing agents, and the whole oxidation process imitated by known free radical processes. Discrepancies between our first results of the reaction between hydroxocobalamin and vitamin B12s can be explained in terms of the pH of the environment. There are indications that linoleic acid free radicals may convert the Fe(II) state of lipoxygenase into the Fe(III) state. Naphthalene partitions between SDS micelles and water with a partition coefficient of 550. Several of these results have been published or are in the press. A general article 'Reactions of free radicals produced from organic compounds in aqueous solution by means of radiation' has been published (A.J. Swallow, Progress in Reaction Kinetics, 1978, 9, 195-366).

593. INFLUENCE OF NEOPLASMS ON HEME OXYGENASE, MICROSOMAL ELECTRON TRANSPORT SYSTEM AND DRUG METABOLISM IN ANIMALS AND CELL CULTURE (ABBREV)

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OBJECTIVE: Heme oxygenase is a microsomal enzyme system found primarily (but not exclusively) in reticuloendothelial cells which catalyzes the conversion of heme to bile pigment. This enzyme system requires NADPH-cytochrome c reductase, a component of the microsomal electron transport system for its activity. The microsomal electron transport system also requires cytochrome P-45 for activity. Microsomal heme oxygenase, cytochrome P-450 and NADPH-cytochrome c reductase have been measured in normal human spleen and in spleen from patients with lymphoproliferative disorders and cancer (J. Lab. Clin. Med. 93, 838-846 (1979). Some malignant states were characterized by increased activity of splenic heme oxygenase (Hodgkin's disease, chronic lymphocytic leukemia, gastrointestinal tract cancer), whereas others were not associated with any significant change in activity (non-Hodgkin's lymphoma, chronic myelocytic leukemia). The specific activity of other microsomal electron transport system components was significantly increased only in chronic lymphocytic leukemia (NADPH-cytochrome c reductase) and Hodgkin's disease (cytochrome P-450). As a result of these findings, a systematic examination of microsomal electron transport system components, heme oxygenase activity, and drug metabolism in Wistar rats bearing Murphy-Sturm lymphosarcoma 2-15 days following I.M. transplantation has been done. Hepatic cytochrome P-450 levels and NADPH-cytochrome c reductase activity decreased significantly and hepatic microsomal oxidative drug metabolism was also significantly diminished. Conversely, hepatic heme oxygenase activity was significantly increased. Splenic microsomal oxidative functions and heme oxygenase did not demonstrate similar changes. Utilizing double label isotope techniques, it was found that the relative rates of cytochrome P-450 synthesis and degradation were both decreased in tumor-bearing rats. This suggests that the capacity for hepatic microsomal oxidative drug metabolism is reduced in Murphy-Sturm lymphosarcoma-bearing rats, primarily due to decreased cytochrome P-450 content as a result of reduced cytochrome P-450 synthesis. The resultant presumed increase in the size of hepatic free heme pool may cause induction of hepatic heme oxygenase. (Text Abridged.)

594. ELECTRON TRANSFER IN MIXED FUNCTION OXIDATIONS - THE MICROSOMAL CYTOCHROME P-450 SYSTEM

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We propose to study the interactions of the redox domains in the liver microsomal mixed function oxidase, NADPH-cytochrome P-450 reductase -cytochrome P-450, a carcinogen-activating system. Specifically we propose to examine the reductase in its holo-, and FMN-depleted forms, fully oxidized, semiquinonoid, and reduced, and chemically modified at tyrosine, tryptophan, and sulfhydryl, in order to determine the extent of interaction between the two component flavins, FAD and FMN, and the distance between them. We plan to employ fluorescence energy transfer, circular dichroism, electron spin resonance, resonance Raman spectroscopy, and optical spectroscopy in this work. We will also determine the amino acid sequence and attempt to crystallize the reductase with a view toward testing the crystals for potential X-ray diffraction analysis. Other physical and chemical probes of the structure of the reductase will be tested in order to throw light upon the mechanisms of intramolecular and intermolecular electron transfer and its regulation during the mixed function oxidase process.

595. MECHANISM-BASED INACTIVATORS OF CYTOCHROME P450

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The microsomal cytochrome (cyt) P450 mono-oxygenase system, found in a variety of mammalian tissues, contains several related isozymes which participate both in detoxification of many drugs and other environmental agents, and in their activation to toxic, mutagenic and carcinogenic forms. Specific inhibition of mono-oxygenase activity can be effected by cyt P450-mediated formation of reactive metabolites which subsequently modify the enzyme covalently with loss of activity. In this study, specific cyt P450 isozymes will be purified from rat liver microsomes and the mechanisms of their inactivation by three drugs will be investigated. In particular, the kinetics of inhibition will be studied to ascertain whether there is a pseudo-first order loss of activity, whether it is irreversible, and whether it occurs concomitnat with formation of a stoichiometric enzyme-inhibitor complex. The chemical nature of any covalent adducts formed with the heme prosthetic group or with the apoprotein will be determined, including the identification of any modified amino acid residues. Structural features which influence the partitioning of metabolically activated compounds between pathways leading to turnover and inactivation will be evaluated by studying a series of chemically related inhibitors. Results obtained from these and other experiments should help elucidate the detailed enzymatic mechanisms of this important drug-metabolizing system, and eventually lead to the design of isozyme-specific cyt P450 inactivators that may be used for specific pharmacological purposes.

596. ENERGY METABOLISM IN CANCER PATIENTS

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This project is designed to quantitatively evaluate energy expenditure and the composition of the fuel mixture burned in patients with gastrointestinal cancer. In addition, glucose kinetics (oxidation rates, pool sized, turnover rates and recycling) will be measured using 14C-U-L-glucose, the degree of gluconeogenesis will be measured using 14C-L-alanine, and whole body protein synthesis and catabolic rates will be measured using 15N-L-alanine. These kinetic studies will be carried out at the beginning of the patient's hospital stay and during nutritional support before and after surgical removal of the tumor. Nutritional support will be provided by parenteral enteral solutions.

The data should provide information on energy and substrate demands of the host and of the tumor by comparing the initial study (post-absorptive) with the pre-op study during nutritional support. Compartmentalization of the host to tumor demands may be ascertained by comparing the results before and after surgical removal of the tumor employing the same nutritional intake baseline in both studies. The post-operative study will be conducted only when the surgical catabolic transients are absent, which will be confirmed by a number of measured responses.

It is hoped that these studies might provide additional information related to the amount and kind of nutritional support cancer patients should receive in their total care.

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597. AUTOREGULATION IN TRANSPLANTABLE TUMORS

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OBJECTIVE: To investigate shifts toward aerobic glycolysis, as the tumor develops, by measuring changes in enzyme levels, cellular proliferation, pH, redox potential and biopotential.

APPROACH AND PROGRESS: In vivo systems which continuously monitor pH, redox potential and biopotentials have been established and an inverse relationship between pH and cellular proliferation with tumor development has been found

598. PATHWAYS OF ENERGY METABOLISM IN MALIGNANCY IN VIVO

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The amino acids and carbohydrates that are oxidized for energy are transported between the tissues and organs by well-defined inter-organ transport cycles. In general, these cycles are arranged so that a metabolic product released from one organ is absorbed and oxidized by another. For example, glutamine released from skeletal muscle into the venous blood is absorbed and oxidized to CO2 by the intestinal mucosa. Recent in vitro experiments have suggested that glutamine and the ketone bodies are the major respiratory fuels for fast-growing, undifferentiated tumors. Mitochondria from these tumors contain a kidney-type, phosphate-dependent glutaminase and an NAD(P)-dependent malic enzyme, the enzymes required for the net oxidation of glutamine, and succinyl CoA:acetoacetate CoA transferase, the enzyme necessary for ketone body oxidation. The activities of these enzymes are low in normal liver and the slow-growing hepatomas but high in fast-growing, undifferentiated hepatomas. Consequently, new enzyme synthesis occurs during tumor progression in hepatomas. We propose that a common pattern of substrate utilization for energy production is adopted by all undifferentiated tumors. The pattern is characterized by the oxidation of glutamine and ketone bodies as the major respiratory fuels. The objective of this research is to test this hypothesis in vivo using a series of transplanted hepatomas and other tumors of varying growth rates and degrees of differentiation. A clearer understanding of energy production in tumors, the changes in energy production that occur during tumor progression, and the inter-relationship between tumor and host will result.

599. PHOSPHORYLATION EVENTS AND TRANSFORMED CELL MEMBRANES

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Many cytoplasmic events are known to be regulated by one or more phosphorylation-dephosphorylation reactions. A primary event in the transformation of chick embryo fibroblasts by Rous sarcoma virus (RSV-CEF) may be the production of a 60K protein possessing phospho-transferase activity (Collett and Erikson, 1978). Recent evidence shows the pp60src to be concentrated on the cytoplasmic surface of the membrane (Willingham et al., 1979).

Our preliminary work has identified two subsets of membrane phosphoproteins and one subset of membrane-associated phosphoprotein showing phosphorylation differences between normal and RSV-transformed chick embryo fibroblasts. The identification and function of these target proteins are not yet clear, nor has the phosphokinase activity of the isolated membranes been fully characterized.

We propose to compare and characterize the endogenous phosphorylating ability of isolated membranes from normal and transformed cells. The assay procedure will be characterized in terms of the total incorporation of phosphate, pH optimum, substrate specificity, the influence of several activators, and product analysis by two-dimensional electrophoresis. Later, column chromatography of solubilized membrane proteins may lead to the isolation of a spectrum of kinase activity, the pattern of which may differ in transformed cells.

We will continue to work on the phosphorylation of intact normal and transformed CEF cells with exogenously added 32Pi, comparing the labeling pattern in these membranes to isolated membranes which have been endogenously labeled with gamma-32P-ATP. Phosphoproteins will be analyzed by two-dimensional gel electrophoresis. Maintenance of topological relationships may direct the phosphorylaton of substrate proteins and a comparison such as this should help to assess the importance of such relationships. Our preliminary findings indicate that this area may be a very fruitful field for future research as distinct labeling patterns are seen.

BIBLIOGRAPHIC REFERENCES: Witt, Daniel P. and Gordon, Julius A. (1980) Specific Dephosphorylation of Membrane Proteins in Rous Sarcoma Virus-Transformed Chick Embryo Fibroblasts. Nature 287, 241-244.

600. PROTON TRANSPORT KINETICS OF RECONSTITUTED CHLOROPLAST COUPLING FACTOR

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The coupling of proton translocation to phosphorylation is a fundamental energy transduction process in biochemistry. Although ATP synthesis is generally believed to be driven by the proton chemiosmotic potential, little is known about the molecular mechanisms involved. This work proposes to study the kinetics and mechanism of proton transport in a reconstituted model system. The dicyclohexylcarbodiimide sensitive ATPase (chloroplast coupling factor) has been actively reconstituted into phospholipid vesicles. This provides a less complicated system that is more amenable to kinetic studies. The proton transport can be studied by introducing a pH indicator into the solution inside the vesicles. The stopped-flow kinetics of proton transport are then monitored by spectroscopic changes in the indicator. The transient kinetics of proton transport will be compared with ATPase activity. The kinetic dependence on pH, pH gradient, and temperature will also be investigated. Thermodynamic and mechanistic information can be derived from such studies.

601. ROLE OF UBIQUINONE-PROTEINS IN BIOENERGETICS

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The aim of this research is to obtain basic information about the molecular mechanism of ubiquinone (Q) in mitochondrial electron transport. It is planned to take dual approaches, namely with and without isolation of functionally active form of Q, i.e. Q-linked to specific proteins (QP-s) as respiratory carriers. There are 3 QP's. For isolation, we plan to apply the principle of the sequential fragmentation of the respiratory chain. The QP involved in the succinate dehydrogenase segment, or QP-S, has been isolated from our cytochrome b-c1 complex, but is required for further improvement. As starting material for isolation of QP-C and QP-N, the QP-S free b-c1 complex and a particulate NADH dehydrogenase will be used, respectively. Mild non-ionic detergent solutions in the presence of dilute hydrogen breaking agent are planned to dissociate QP-C and QP-N. After dissociation, conventional methods of enzyme isolation are planned for preliminary purification and newer techniques are slated for the final steps. Various usual and newly developed physico-chemical methods will be applied for characterization. The final substantiation of the locations of QP's in the sequence must rely on physical reconstitution to restore the parent functionality.

602. PORPHYRIN SPECIFICITY IN MALIGNANCY

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Various porphyrins are being studied in an attempt to understand why some of them show a relatively high accumulation and/or retention in malignant tissue. Using autoradiographic and fluorescent methods, we find that hematoporphyrin derivative (Hpd) accumulates in the mouse in the following order: liver-kidney-spleen-lung-tumor (SMT-F)-skin-muscle. However, the intratissue distribution changes with time after injection in all tissues except the tumor which maintains a 5 to 1 preference for porphyrin retention in the stroma for at least 72 hours. Normal tissues clear Hpd as plasma levels drop (half-life equals 3 hours). However, even in normal tissues, stromal preference is apparent at clearance since these areas clear more slowly.

Hematoporphyrin (HP), however, shows no tendency for intracellular accumulation in any tissues, normal or malignant, but is retained mainly in the stroma. Its clearance from tissue is more rapid than that of HpD.

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Goldfarb, A. Photoradiation in the treatment of recurrent breast carcinoma. J. Natl. Cancer Inst., 62(2):231-237 (February 1979).

603. BIOLOGIC STUDIES WITH LIVER PEROXISOME PROLIFERATORS

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The purpose is to pursue investigations to delineate the acute and long-term effects of hypolipidemic hepatic peroxisome proliferators, in an attempt to understand the biologic significance of peroxisomes in general and peroxisome proliferation in particular. We will expend considerable effort toward evaluating biologic events associated with hepatic peroxisome proliferation. These studies will include: screening of selected chemicals of unusual structure with potent hypolipidemic activity for induction of peroxisome proliferation and peroxisome-associated enzymes in hepatic and extrahepatic cells, characterization of peroxisome membrane by freeze-fracturing, purification and subcellular localization of 80,000 MW protein induced in liver by hepatic perosisome proliferators, and induction and degradation of peroxisomes and peroxisome-associated enzymes in vitro in isolated liver cells. In addition, the proposed research will also strive to establish, as well as elucidate, the role, if any of persistent peroxisome proliferation and hepatomegaly induced by the hepatic peroxisome proliferators in the initiation and promotion of hepatocellular carcinomas in rats. The information obtained from these studies should further our knowledge of peroxisomes as well as allow us to recognize the implications of the multiplicity of biologic effects of peroxisome proliferators.

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604. THE GLUTAREDOXIN AND THIOREDOXIN SYSTEMS IN CARCINOGENESIS AND TUMOR GROWTH

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OBJECTIVE: To relate the activity of two major thiol-disulfide interchange systems to transformation into the malignant growth state and its propagation. We are studying the thioredoxin and glutaredoxin systems in different cells in order to find basic regulatory functions for thioredoxin and glutathione. A hypothesis that the defective regulation of growth in tumor cells is caused by a defective interplay between thioredoxin and glutathione leading to uncontrolled DNA synthesis is tested.

APPROACH: Measurements of oxidation state of thioredoxin in rat liver cells and hepatoma cells. Studies of the concentrations and activities of GSH, thioredoxin, thioredoxin reductase, glutathione reductase in normal and tumor cells. Purification and characterization of glutaredoxin from calf thymus cells. Characterization of the function of E. coli thioredoxin as a subunit in phage T7 DNA polymerase required for virus DNA replication.

605. ROLE OF DISULFIDES IN NORMAL AND TUMOR CELL GROWTH

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Many diverse observations suggest that extracellular, as well as intracellular, thiol-disulfide reactions are associated with cell growth control and that viral cell transformation results in changes in such phenomena. A major hindrance to further investigations of the importance of such observations is the lack of a quantitative, sensitive, and specific analytical method for analysis of low molecular weight thiol-disulfide components. The specific aims of the present studies are: (1) to develop a general method for analysis of thiols, and thereby disulfide components, based upon isolation of thiols by affinity to a Sepharose-linked mercurial, conversion of the thiols to mixed disulfides of cystine, and quantitative measurement of the latter by conventional amino acid analysis; (2) to utilize this methodology to analyze the thiol, disulfide, and protein mixed disulfide content of two lines of normal cells (3T3 and MDCK), of two corresponding lines of virally transformed cells (SV40-3T3 and MSV-MDCK), and, simultaneously, of the growth media during exponential growth at low cell density and during growth at high cell density (confluent cells).

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606. ABNORMAL HISTAMINE METABOLISM IN EXPERIMENTAL TUMORS

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We studied different biochemical parameters of the histamine metabolism of a transplantable syngeneic spontaneous murine mammary adenocarcinoma with a high degree of non-differentiation. The endogenous histamine content is significantly higher than that of intestine and lung, taken as normal tissue.

This result is not due, in this case, to an increase of the histamine biosynthetic capacity, which is smaller than that of lung. Chronic treatment with 0.1 mg/kg body weight of histamine during seven days significantly decreases the biosynthesis of histamine in the tumor without affecting its endogenous histamine content. In the intestine and lung no significant modification of histamine biosynthesis is observed. The concentration of radioactivity in the nuclear fraction of the tumor is significantly higher than in intestine after incubating either tissue with 3H-histamine.

We are presently studying the influence of histamine and its metabolites, as well as indole acetic acid, on the growth and induction of inducible tumors.

607. CHARACTERIZATION OF BIOLOGICAL TISSUES BY NMR

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High resolution PMR signals from specific lipids, carbohydrates, and proteins have been observed in normal, carcinogen-damaged, and cancerous tissues. These signals are indicators of the chemistry of the metabolic processes taking place in these tissues. Consequently they represent a new set of descriptors for normal, carcinogen-damaged, and cancerous tissues. A detailed study of these signals will be made using normal rat liver, carcinogen-damaged liver, hepatoma, normal rat mammary, lactating mammary, pregnant mammary, mammary tumors, and normal and leukemic mouse leukocytes. Concurrent with the PMR studies detailed chemical analyses for carbohydrates and lipids will be carried out in order to identify the observable PMR signals. It will be determined whether these signals can be used to measure pool sizes of specific lipids and carbohydrates in the tissues. It will be determined whether monitoring these PMR signals can be used as a means of studying the kinetics of changes in lipid and carbohydrate pool sizes in intact cells and excised tissues. Carbon-13 NMR studies will be made on the compounds isolated from the tissues in order to confirm the studies deduced from PMR spectra. NMR techniques to be used for proton measurements include pulsed Fourier transform and rapid scan correlation approaches.

608. THE METABOLISM OF NORMAL AND LEUKEMIC LEUKOCYTES

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Current experiments are concerned with: (1) biochemical mechanisms in the Killmann experiment: a critique of the 'deoxyuridine suppression test'; (2) development of a sensitive enzymatic assay for picomole quantities to dTMP in tissue extracts; (3) studies on the incorporation of uracil into DNA; (4) studies of one-carbon metabolism of normal and leukemic leukocytes;

(5) studies of folate and vitamin B12 uptake in cultured lymphocytes; (6) development of a new assay for folylpolyglutamate synthetase and partial purification of the enzyme from rat liver; (7) binding of human transcobalamins to rat liver cell fractions; and (8) studies of the microbiological (Lactobacillus leichmannii) assay for serum vitamin B12: characterization of serum vitamin B12 analogs that are not detected by the microorganism.

609. POLYCYCLIC AROMATIC HYDROCARBONS - PROTEIN INTERACTIONS

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This research is intended to obtain information concerning carcinogenic hydrocarbon-protein interactions which play an important role in the carcinogenicity of these hydrocarbons. The activation of water-insoluble hydrocarbons require their binding to hydrophobic proteins, followed by catalyzed oxidation in association with the proteins. However, only limited information is available for the mechanism of these interactions. In the studies, the involvement of reactive SH-groups, know to produce both superoxide and thiyl radicals, in the modification of carcinogenic hydrocarbons is studied using techniques such as fluorescence and electron spin resonance spectroscopy. We find that carcinogenic hydrocarbons are chemically modified in interaction with the SH-protein serum albumin or the SH-amino acid cysteine, producing various products (oxidized products, radical species or UV-fluorescent products). These hydrocarbons from environmental pollutants are taken up by the skin, lung and blood, bound to proteins, activated to reactive forms and transferred to nucleic acids. Thus the research is relevant to energy-related health problems.

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610. BIOCHEMICAL CHARACTERISTICS OF NEOPLASMS (RODENTS, DOGS, HUMAN)

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This is part of a broader project. A summary of this subproject is not available.

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Westwood Building, Room 10A18
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If you have already submitted a description of your research to the Smithsonian Science Information Exchange (SSIE), to the Clearinghouse for Ongoing Research in Cancer Epidemiology in Lyon, France, or to the Current Cancer Research Project Analysis Center (CCRESPAC) within the past eight (8) months, please do not fill out this form.

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